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PROJECT NO. B-106

AN INVESTIGATION OF THE EFFECTS OF MINUTE QUANTITIES OF CHEMICAL VAPORS ON AIR-BORNE BACTERIA

BY

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SUMMARY

The specific aims of this project were to determine the effect of various chemical vapors on certain bacteria, to correlate the bactericidal effects with the molecular properties of the vapor, and to develop a scale of bactericidal effectiveness of vapors similar to the "phenol number" used for disinfectants. These aims have been achieved utilizing only a single bacterial species. Comparison with the results of other workers indicated that the principles elucidated are generally applicable to other bacterial species. This report gives the results obtained from studies of the effects of a number of chemical vapors on air-borne bacteria; the aerial disinfectant properties of these chemicals as related to their in vitro toxicity in terms of fractional saturation; and specific conditions, test organism, equipment, and reference chemicals for the establishment of a scale of effectiveness of aerial disinfectants.

In the course of the studies, various items of equipment have been developed. Basic studies have been carried out dealing with the nature of the air-borne bacterial particle, the effect of relative humidity on the survival of air-borne bacteria in the presence of both controlled room air and chemical vapors, and the factors involved in the sampling of air-borne bacteria.

SIGNIFICANT ACCOMPLISHMENTS

Techniques and procedures were developed for experimental aerobiology which can be used readily by other workers to form the basis for a standard procedure for the evaluation of aerial disinfectants. These techniques and procedures include an aerosol chamber for carrying out detailed studies on air-borne bacteria, including the estimation of particle size, and also aerosol cylinders for the rapid screening of candidate compounds as aerial disinfectants.

Evidence as to the nature of air-borne bacterial particles and the importance of the nonliving material associated with these particles in forming the immediate environment of the air-borne bacteria is shown.

The significant role of relative humidity in determining the survival of air-borne bacteria, through its effect upon the immediate environment of the bacteria, both in respect to natural survival and in determining the ultimate effect of aerial disinfectants on air-borne bacteria is presented.

The relation between *in vitro* bactericidal power and aerial disinfection power of both hygroscopic and nonhygroscopic compounds at varying degrees of aerial saturation and under various conditions of relative humidity were equated. The equated relationships suggest that the standard evaluation of bactericides should be in terms of the die-away constant *k* because both *in vitro* and aerial killing power can be expressed in this manner.

A detailed study of the method of action of critical-orifice, liquid impingers is presented. Although this study was not completed, sufficient evidence has been collected to form the basis for future studies which might lead to the development of a high volume air sampler of this type.

Evidence is presented which helps to resolve certain disagreements expressed in previous literature concerning the relative effect of low and high humidity on the survival of air-borne bacteria, the existence of a "critical" rate of drying of organisms dispersed into the air, and whether glycols act as aerial disinfectants through sedimentation or through toxicity.

The inadequacy of intra-experimental reproducibility as a criterion for the evaluation of the usefulness of samplers for aerobiology was demonstrated. This was shown by the 2:1 ratio of enriched to plain-gelatin fluid in the recovery of viable organisms, resulting in large errors in the estimation of particle size.

That thermal precipitation can be applied successfully to the sampling of bacterial aerosols has been demonstrated. Although the full possibilities of the sampler† developed were not exploited in connection with this project, it has proven to be a valuable instrument in the sampling of air for particulate matter of all kinds.

† Kethley, T.W., Gordon, M.T., and Orr, Clyde, Jr., "A Thermal Precipitator for Aerobiology." Science 116, 368 (1952).

I. PREFACE

The primary aim of this work was to determine the effect of small amounts of chemical vapors on the viability of air-borne microorganisms. In order to approach this objective, it was necessary to establish techniques, to devise equipment, and to make certain basic investigations. It was also necessary to undertake a partial evaluation of existing literature because of the implicit conflicts within the literature and the apparent conflict of some of our findings with those of other workers. In terms of volume of work, these preliminary and auxiliary studies have made up the majority of the effort during the past several years. However, as a result of these efforts, it has been possible to evaluate the effect of a number of different types of chemical vapors on the viability of air-borne bacteria. Mainly, the data from these studies were correlated with the in vitro bactericidal activity of these compounds, and approximation equations are offered for these relations. Details are presented of relatively simple and reproducible methods for the evaluation of the effect of chemical vapors on air-borne bacteria.

This report will, therefore, review the present knowledge of the problem, present reliable methods and techniques for future studies, enumerate those variables producing significant effects, recommend avenues for further study, and present approximation equations for translating in vitro bactericidal activity into terms of aerial bactericidal activity.

This report could serve as a basis for further work on the part of other investigators, should reconcile some of the divergent views expressed in the existing literature, and could be used as a handbook in the field of aerobiology. For this reason, precise and detailed accounts of methods, equipment, and literature discussions are included in more detail than would normally be expected.

II. HISTORICAL BACKGROUND

An extensive literature exists relating to the general subject of aerobiology and the specific problems pertinent to the study of the effect of chemical vapors on air-borne organisms. A literature survey has been made, and abstracts have been prepared and filed at the Georgia Institute of Technology. The methods and procedures of the majority of other workers can be found in one or more of the published volumes.† Because of the availability of these books, no attempt is made to discuss them in detail. However, as the program developed, it became apparent that some understanding of the mechanism of the death of air-borne organisms was essential not only for planning but also for interpreting the results obtained. Therefore, the conclusions reached by these workers as to the mechanism of death were studied and are presented here as the background for the studies.

Since the field of experimental aerobiology is complex and elaborate equipment is essential, only a relatively few groups have made major contributions to this field. In selecting material published by these various groups only those articles pertaining to the mechanics of the death of air-borne organisms were chosen. The bibliography of this report is therefore representative, but not necessarily comprehensive insofar as authors are concerned.

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A. Mechanism of Transfer of Lethal Agents from the Air to the Bacterial Particle†

In general, it is the concensus of opinion that lethal agents reach the bacterial particle by condensing on that particle (1-8, 14). Although many workers originally believed that transfer was accomplished by contact between droplets of the toxic substance and the bacterial particles, more recent work has demonstrated that this is highly improbable. Not only has it been shown that vapors of the active agents are effective in the absence of droplets of the agent, but also it has been shown that, under conditions usually existing, it is improbable that liquid droplets will coalesce unless they are greater than 50 microns in diameter. In those instances where the lethal agent exhibits a very low vapor pressure and where experiments have shown that dispersions of that agent in the air are ordinarily more effective than the vapors alone, Nash (8) has shown that distillation from the droplet of the agent to the bacterial particle is the most probable method of transfer.

Although the above-mentioned studies appear to prove conclusively that the probable mechanism of transfer of lethal agents in the air to bacterial particles is by condensation of the vapors of the agents onto the particles, no experimental proof has yet been presented which absolutely precludes other methods of transfer such as adsorption or contact with very small droplets existing in high population densities.

B. Mechanism of Air Disinfection by Chemicals (Other than Water)

Assuming that the bacterial particle receives a dose of the disinfecting agent, air disinfection can be accomplished in the following ways: lethal action similar to that of disinfection *in vitro*; enlargement of the particles, causing an increase in the settling of the bacterial

† See bibliography at the end of this chapter.

particles from the air; setting up a chain of events which adversely affect the bacteria, already in an unfavorable medium, which prevents the subsequent growth of the bacteria, even though collected from the air and placed in more favorable circumstances; and dehydration or mechanical effects, particularly on the cell wall. Obviously a combination of these, as well as a single mechanism, could be responsible for aerial disinfection.

1. Lethal Action Identical with In Vitro Action

The majority of English and American workers support the theory that lethal action in the air is identical with *in vitro* action.(4,6,7,8,9,10) The greatest single objection to this theory has been the discrepancy between aerial and aqueous concentrations of agents required to accomplish disinfection in the air or in the test tube, respectively.(14) Many agents of little value as bactericides *in vitro* have proven to be effective disinfectants in the air, and, conversely, effective bactericides *in vitro* have proven to be worthless in air disinfection. Further, interrelationships within a homologous series have not always been consistent in air disinfection, and varying concentrations have not always given variations in kill consistent with predictions. Several recent reports have presented evidence and suggested methods whereby existing data may be correlated (7,8,9,10) by taking into consideration the differences between the state of the environment and the state of the bacteria in comparing results from air disinfection experiments and those from *in vitro* experiments. In the reports just referred to, it is postulated that physical factors are of primary importance in determining the effectiveness of a particular agent as an air disinfectant.

One of the most important physical factors in determining the effectiveness of an aerial bactericide is the condition of the bacterial particle--its size, content of moisture, water-soluble solids, and organic matter. It is considered that this factor will determine the final concentration of air disinfectant in the particle and the rate of diffusion of the chemical through the surface of the particle to the bacteria themselves. The rate of kill will vary inversely with the square of the diameter of the bacterial particle.(7) Under any particular condition of humidity in the air, the moisture content and the water-soluble solid content of the bacterial particles are interrelated in that the moisture content of the particle is probably a function of the aqueous tension of a saturated solution of the most soluble component.(10) However, the large increase in size of a bacterial particle with increasing relative humidity as reported by other workers(7) would indicate that the organic matter of the particle plays a large part in determining the water content of the particle. The water content of a particle, all other factors being equal, affects the final concentration of the toxic agent; the more moisture, the less concentrated the agents(7) (although, in the case of hygroscopic agents this might not be the case) (10). The amount of organic matter associated with the bacterial particle will determine the inward diffusion rate of the toxic agent to the bacteria.(7)

Another important physical factor in determining the effectiveness of an aerial bactericide is the relative humidity of the air. Assuming that the kill is proportional to the concentration of the toxic agent in the air, for those compounds which must be used at nearly saturation, the relative humidity determines the actual concentration in the air.(10) Further, it has

been suggested, for compounds not used at saturation, that the "effective," concentration, i.e., the concentration of the toxic agent present in the water associated with the bacteria, is determined by the relative humidity.(9) As indicated previously, the moisture content of the bacterial particle will be affected by the relative humidity. It is generally accepted that certain values of relative humidity adversely affect bacteria in the air and, thereby, give rise to variations in the apparent effectiveness of certain air disinfectants. This has been explained by postulating the existence of a "critical" degree of moisture content at which a bacterial cell becomes much more susceptible to toxic agents than when the cell contains more or less water.(11)

The nature and concentration of the toxic agent is also important in determining the effectiveness of an aerial bactericide. It is on this point that the greatest disagreement exists. The difficulty of interpreting "effective" concentrations and the differing results obtained at low and high humidities with various classes of compounds has made the problem of comparing various compounds at various concentrations very extensive. It is pointed out that phenols, none of which is very soluble in air, exhibit a high degree of activity as air disinfectants.(4) The proponents of the glycols include "high hygroscopicity" as an essential requirement of a useful air disinfectant.(10) Another group (7) considers high water solubility as a requirement for maximum air-disinfection action.

Although the probable differences between concentration in the air and effective concentration in the bacterial particles have led many workers to conclude that there is not necessarily any relationship between in vitro bactericides and aerial disinfectants, it would seem likely that a more

thorough understanding of the physical factors involved will lead to a reconciliation of such data. It is also likely that the more complex conditions existing in the air as contrasted with *in vitro* tests can explain apparent discrepancies in the variation of kill with varying concentrations of toxic agents in the air.

2. Disinfection Caused by Increased Settling Rate

The possibility of disinfecting air by rapid removal of bacterial particles by increasing the size due to condensation of chemical vapors on the particles has been advanced to account for the action of certain glycols (3,7). From the reported data, these conditions can exist when the agent employed is hygroscopic, has a very low vapor pressure, and a very low bactericidal value *in vitro*. This is true for certain glycols, and it may be that an increased settling rate accounts for at least part of the aerial-disinfection activity of these compounds. Particular reference should be made to work on build-up techniques for aerosols (12) whereby it has been shown that ethylene glycol vapor or water droplets can increase the diameter of very small particles to 5 microns or more.

3. Attenuation of the Bacteria in the Air

Little consideration has been given this aspect of the problem, other than to suggest it, (4, 7, 14) although most workers have given some consideration to bacteriostatic action in their sampling studies. The possibility should be kept in mind in view of the lack of definitive evidence clearly excluding it as a factor in air disinfection.

4. Other Factors

A current summation proposes arguments in favor of the importance of dehydration in determining the lethal effectiveness of compounds against airborne organisms (14).

The question of whether or not some mechanical damage, especially to the cell wall of the bacterium, enters into the disinfection of the air is a highly conjectural one. Numerous other factors may be of importance in the mechanics of the death of bacteria in air, but the present evidence indicates that the most probable ones are included in those listed above. It would not seem reasonable to seek alternative paths until these factors have been exhaustively explored.

C. The Effect of Relative Humidity on the Survival of Bacteria in the Air

Aside from its modifying effect on the action of chemical disinfectants on air-borne bacteria, relative humidity has been reported to have a definite effect on bacteria in air supposedly free of toxic chemical vapors. Although sometimes variously reported, the prevailing concept is that relative humidities of 40 to 60 per cent are more unfavorable to bacteria than those above or below this range. A suggested explanation of this phenomenon (11) is that as bacteria are projected into the air from aqueous suspension (natural or experimental), evaporation of water takes place very rapidly from the smaller droplets. This means that the original droplet forming the environment of the contained bacteria is losing water, resulting in a concentration of solutes in that environment, and, at the same time, the bacteria are also losing water. On the basis of this, it is suggested that the rates at which these two processes progress determine the final fate of the bacteria. If the concentration of the solutes in the droplet becomes excessive, the enclosed bacteria (or bacterium) may be adversely affected. On the other hand, it is postulated that if the bacteria dry out rapidly enough to a low moisture content, they become resistant to such adverse effects. Thus a "critical" degree of moisture content for the cell is proposed, and,

presumably, a "critical" ratio of drying rates between the droplet and the bacteria is implied.

Some light has been shed on the possible adverse effect of high concentration of solutes in investigations relating to sodium chloride concentration in air-borne droplets.(11, 13) By diminishing the salt concentration of the aqueous suspension from which the organisms were sprayed, the adverse effect of 40 to 60 per cent relative humidity was decreased. However, insufficient evidence is available at the present time to permit the proper evaluation of these data.

In general, the anomalous effect of relative humidity on air-borne bacteria appears to be the most baffling problem presented by the studies on aerial disinfection. If the suggestions of many of the workers that aerial disinfection actually takes place in the same manner as in aqueous suspensions can be verified by a proper evaluation of the physical factors involved, the peculiar effect of relative humdidity *per se* will be left unexplained. A similar situation exists in the study of the effect of low temperatures on bacterial suspensions where physical factors involving water balance and protective films seem to be of great importance.

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III. EQUIPMENT

A. General Description

The temperature-humidity laboratory used to carry out the aerosol work is a heavily insulated room 12 by 30 feet, 6.5 feet high. The room is divided into two sections--the larger serving as the workroom and the smaller serving as the control room for regulating the temperature and humidity. Figure 1 is a photograph showing the interior of the laboratory as observed from the entrance of the workroom looking back into the control room. On the right, the main aerosol chamber, the outer unit of the aerosol cylinders, and the meter chamber are shown. The conditioned-air entrance to the workroom is located over the opening into the control room with the remainder of the opening serving both as an air return and as a passageway between the two rooms. On the left, the instrument for recording the dew-point and dry-bulb temperatures, as well as tables that provide desirable work space for handling plates, samplers, test instruments, etc. are shown. The wet-test meter used in calibrating critical-orifice impingers, nozzles, and flow meters is located on the table in the foreground.

The interior surface of the walls and ceiling of the laboratory is enameled metal. The floor of the workroom is varnished wood, and the floor in the control room is watertight Masonite. These surfaces can be readily cleaned with a good detergent and water. Water, air, steam, vacuum, and electrical services are provided where needed. Also, the construction of the walls is such that openings for conduit and ducts are easily made.

The temperature range for the workroom can be controlled from 0° to 95° F, and the dew point of the air in the room can be controlled from 25° to 95° F,

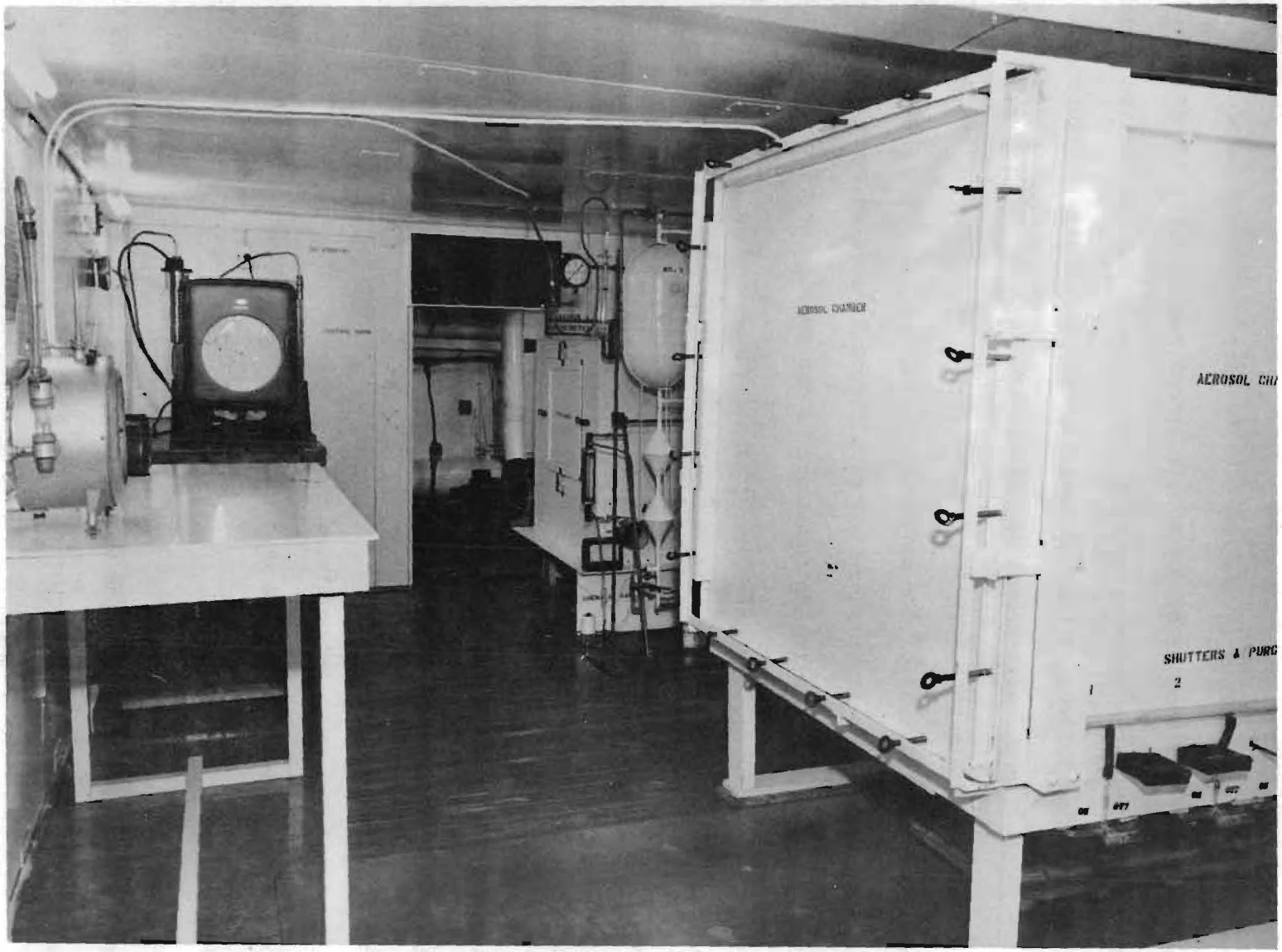


Figure 1. Interior of Workroom.

resulting in a wide range of temperature and humidity (controllable within 1° F for both dry-bulb and dew-point temperatures). The actual conditioning of the air is carried out in the control room. The interior of the control room as viewed from the workroom is shown in Figure 2. The cold evaporators, water reservoir, and pump which circulates the water over the evaporator coils, and the heat exchanger with blower may be readily identified. An inlet for fresh air, though not shown, is located to the right of evaporator No. 2. Also, a steam jet for injecting live steam into the heat blower system for humidification is not discernible. The treated air leaves at the top of the two evaporators through the large ducts along the ceiling which converge just prior to entering the workroom. The blowers in the evaporators combined with the heat blower circulate the air into and from the workroom at about 3,000 cfm. Approximately 60 cfm of fresh, filtered air is continually pumped into the control room through a stack having access to a point outside and above the research building. This air is vented by the aerosol chamber during dynamic runs and through relief shutters or by the meter chamber exhaust system when the chamber pump is not operating. This small amount of makeup air is generally sufficient for comfort because operating personnel in the workroom are kept to a minimum and smoking is prohibited within the room. The desired condition of temperature and humidity is set up by adjusting the temperature of the circulating water, the wet-bulb thermostat which controls the dampers regulating the per cent of air drawn through the evaporator coils, and the dry-bulb thermostat which controls the flow of steam through the heat-exchanger coils. These controls are only semiautomatic until the set point is achieved; intermittent attention is required during the first hour or so

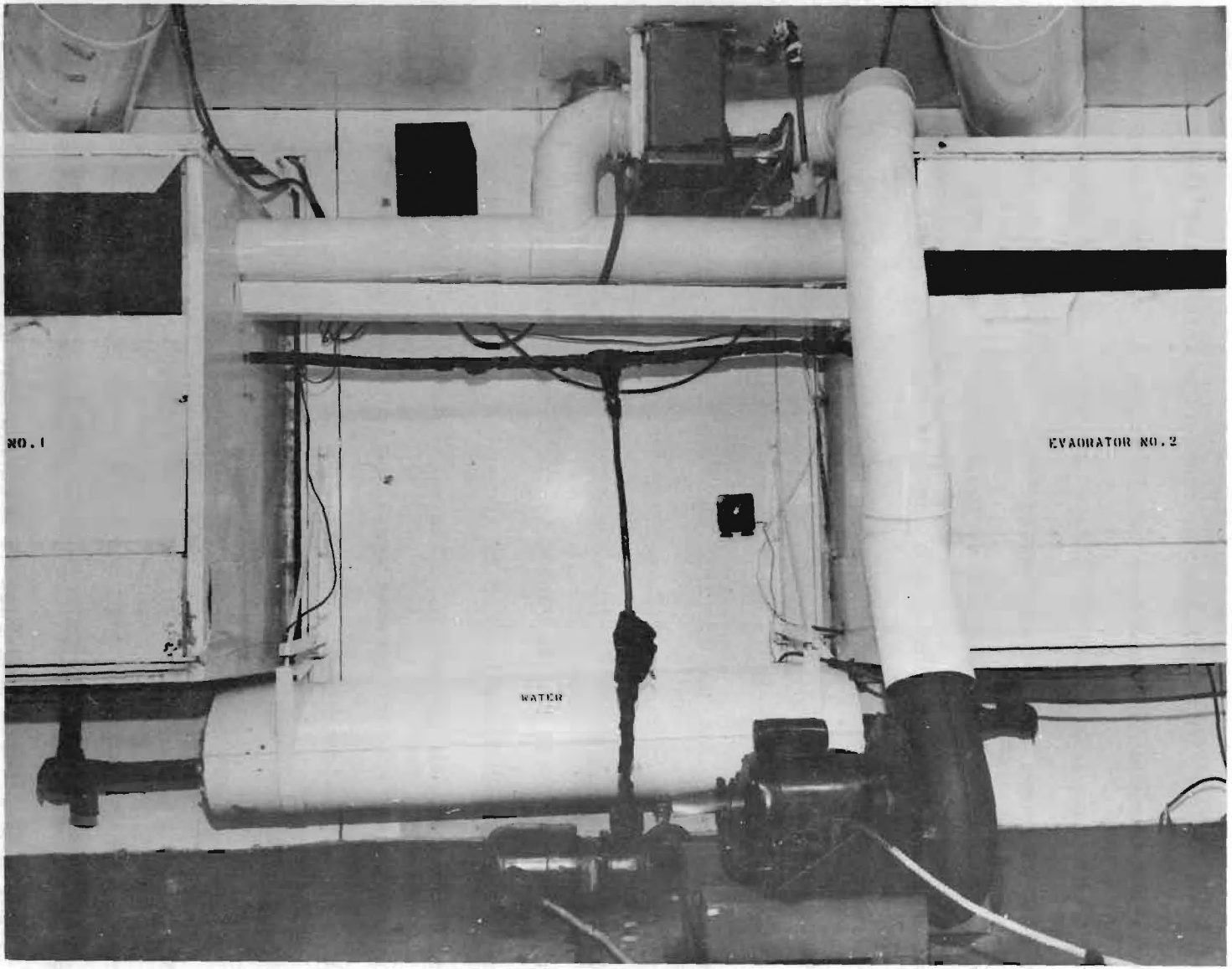


Figure 2. Control Room.

of operation as the controls must be adjusted in relation to the ambient temperature in the building and the temperature and dew point of the outside air. For the higher humidities, live steam is injected at a constant rate; for the lower humidities and temperatures, the fresh-air intake may be closed off.

The aerosol chamber as seen in Figure 1 is located in the right corner of the workroom, about 3 feet from the side walls, and is about 6 inches from the ceiling and 18 inches from the floor. Since the air in the room is circulated at approximately 3,000 cfm, the narrow spacing between the top of the chamber and the ceiling of the room is no deterrent to adequate heat transfer between the room and the chamber.

The chamber itself is a 4-foot cube constructed of tempered Masonite, and one side--the front--is removable. There are no internal projections in the chamber other than the air diffuser. It is 5 inches in diameter and projects from the center of the ceiling into the chamber about 2 inches. The internal surfaces are finished with several coats of a white alkyd resin enamel, each coat hand rubbed to a mirror finish. There are 17 openings in the chamber walls: ten 3/4-inch holes in the left wall for taking air samples; one 3/4-inch hole in the rear wall for connecting a manometer; one 3-inch hole in the center of the ceiling for the air inlet; five 4-inch holes equally spread along a diagonal of the chamber floor, the center one serving as the air outlet, and the remainder are ports for settling samples. The interior of the chamber as viewed with the front side removed is shown in Figure 3. The various sampling ports, outlet hole, and diffuser inlet may be readily identified. Also shown is the frame and screw clamp assembly required for closing tightly the removable side.

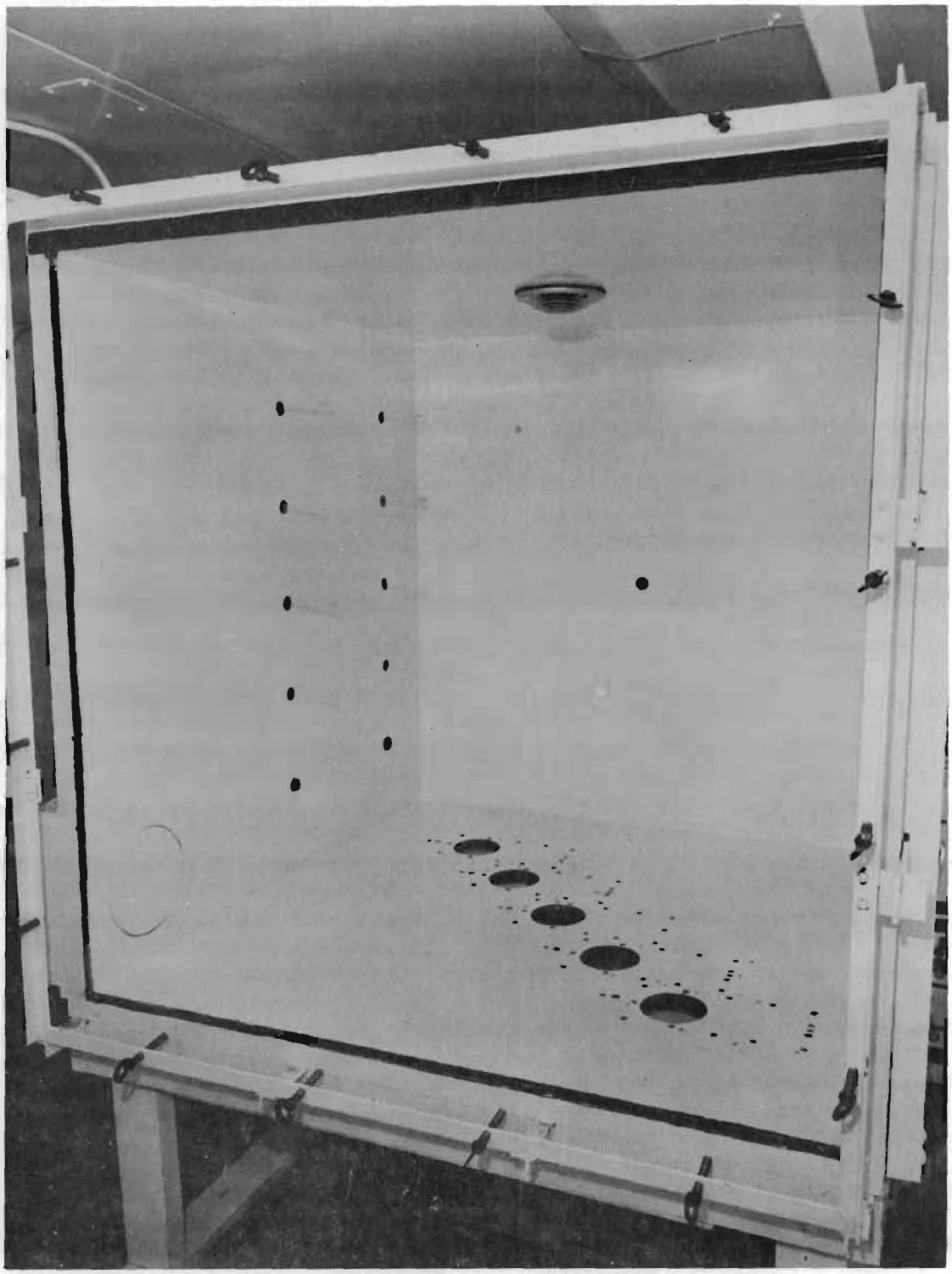


Figure 3. Interior of Aerosol Chamber

A schematic drawing of the chamber and atomizing equipment is shown in Figure 4. During the dynamic runs, the main air stream of the chamber (60 cfm) is drawn from the workroom through filter A and is metered by orifice C, passing through Anemostat D (trade-mark of the Anemostat Corporation of America) and is discharged outside the workroom through filter F. The pressure within the chamber is balanced to that of the surrounding room with the by-pass damper arrangement G, the pressure being read on a manometer.

Clean, regulated, compressed air for the atomizer is metered at I and disperses the bacterial suspension contained in the atomizer J (DeVilbiss No. 40, operated at 6.4 l./min at 20 psi). The resultant aerosol suspension is forced into the 20-inch cubical prechamber K, where the large particles drop out. The air-borne bacteria are thoroughly mixed with the main air stream in the turbulence created at the orifice meter, and then they are uniformly distributed throughout the chamber by the Anemostat. Air samples are then taken from any of the ports indicated for that purpose. Figure 5 shows a number of liquid impinger samplers, which are connected to the air sampling tubes in the side of the chamber and also to the vacuum manifolds. The holders for the samplers slide up and down on the vertical support rods to facilitate the spacing of the samplers and to handle variously sized samplers. (Each individual pump pulls approximately 2 cfm at 23 inches of mercury.) The two vacuum manifolds may be interconnected or used separately with independent vacuum pumps depending on the desired arrangement. Figure 6 is quite similar to Figure 5 but shows the use of heated jackets for controlling sampler temperatures. Although not very discernible in the photograph, these jackets consist of a 6-inch plastic tube with a heating element of resistance wire

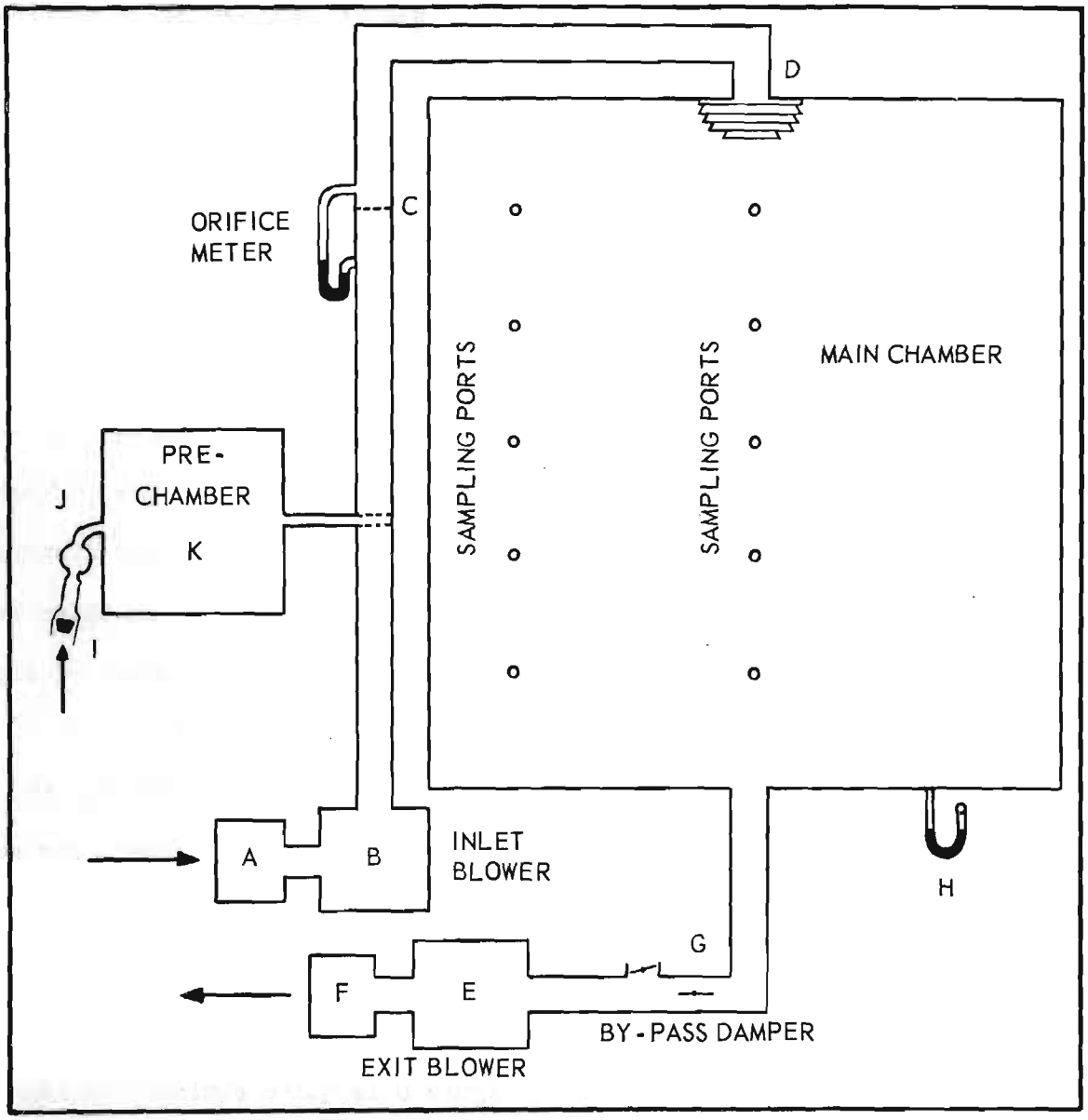


Figure 4. Schematic Drawing of Chamber and Atomizing Equipment.

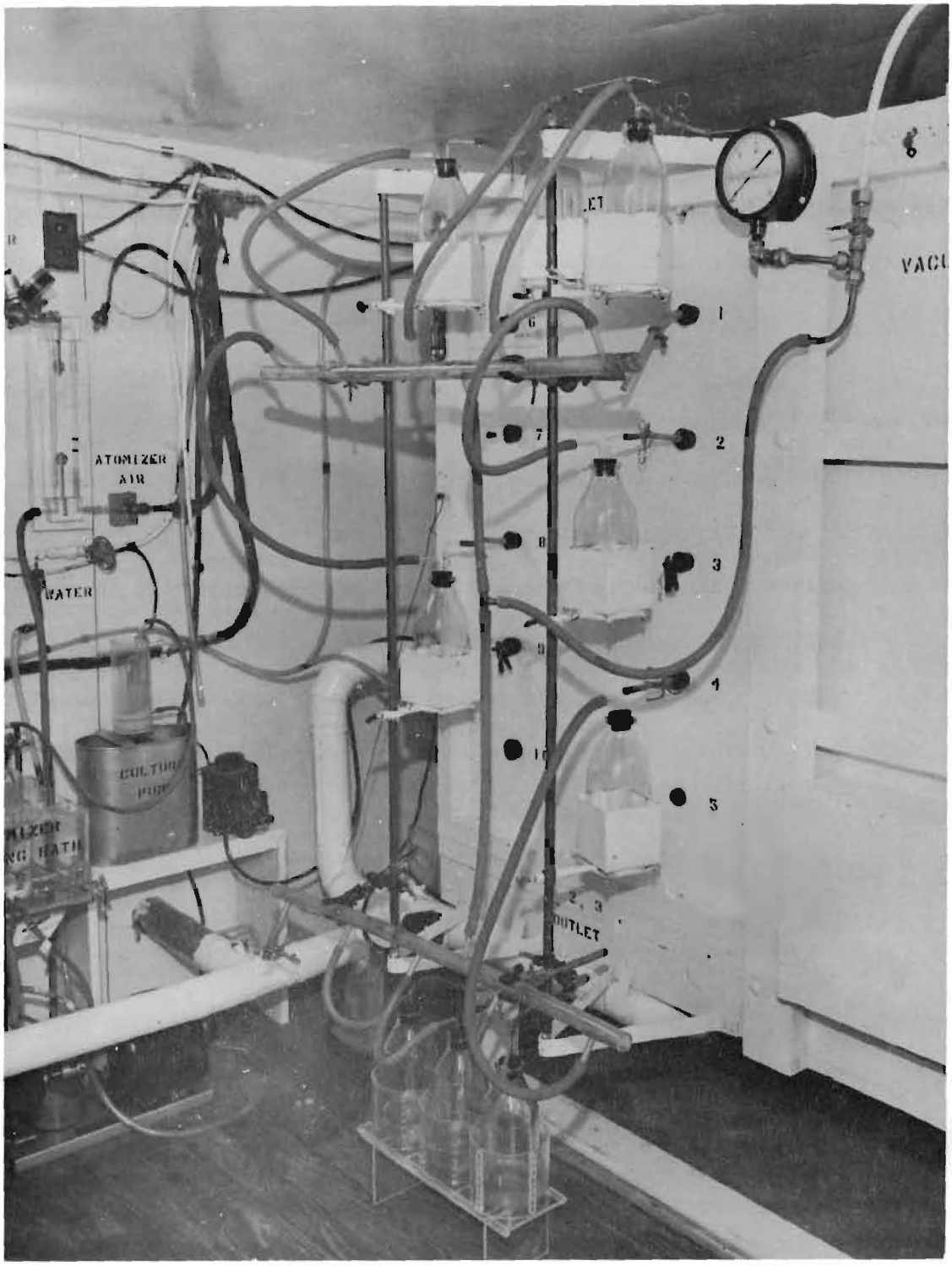


Figure 5. Samplers in Position at Aerosol Chamber.

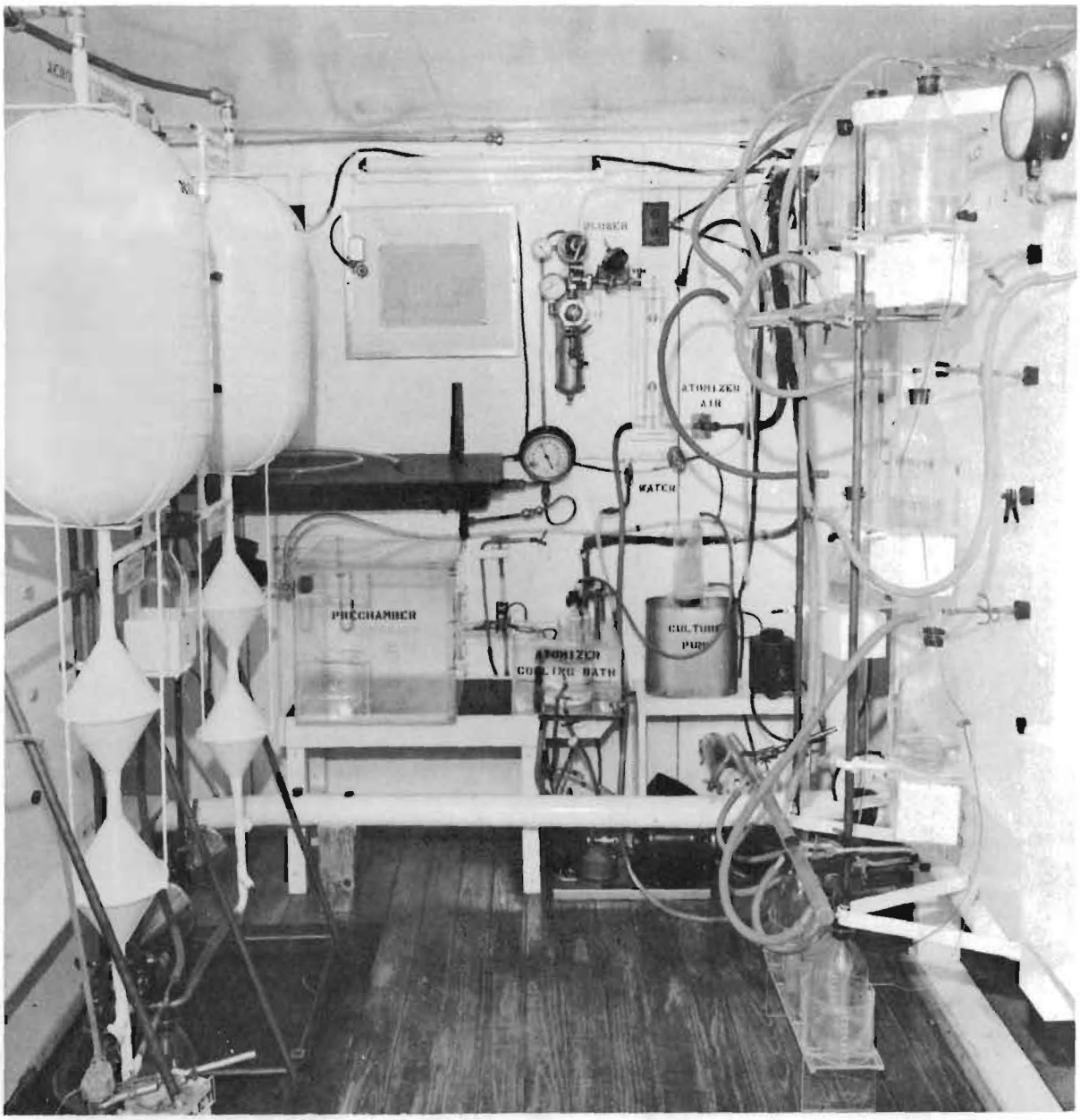


Figure 6. General View of Samplers, Atomizer, and Cylinders.

offset about 1/2 inch within the inner circumference of the tube. Extension tops and felt covers may be added to these jackets when it is necessary to insulate and heat the entire outside surface of the samplers.

The details of the assembly used for air sampling are shown in Figure 7. In use, the milk bottle containing the sampler fluid is sterilized as shown in the center of the center of this photograph. A sterile impinger assembly is inserted just prior to sampling.

The equipment for atomizing the bacterial suspension can be seen in the central background of Figure 6. In order to minimize changes in the bacterial culture during long runs, the atomizer and the 5-inch diameter aspirator bottle used as a reservoir for the culture (see Figure 8) are immersed in a small water bath, the temperature of which is maintained at 45° F. Because the reservoir bottle has a much greater cross-sectional diameter than the atomizer, the level of the culture in the atomizer does not change appreciably for several hours, and it is possible to operate for as long as 10 hours by slightly adjusting the level of the reservoir bottle every 2 hours. Also, to minimize concentration effects within the atomizer, a siphon pump is connected to the atomizer-reservoir system which keeps the culture in the atomizer and that in the reservoir continually mixing with each other. As seen in Figure 6, the atomizing air is metered by the flowmeter just beneath the blower switch before going to the atomizer in the cooling bath. The output of the atomizer flows into the prechamber from which it can then either be directed to a throw-away filter system, to the aerosol chamber on the right, or to the aerosol cylinders on the left, depending upon the situation desired. The regulator to the left of the blower switch controls the air for the settling-plate holder, air-purging

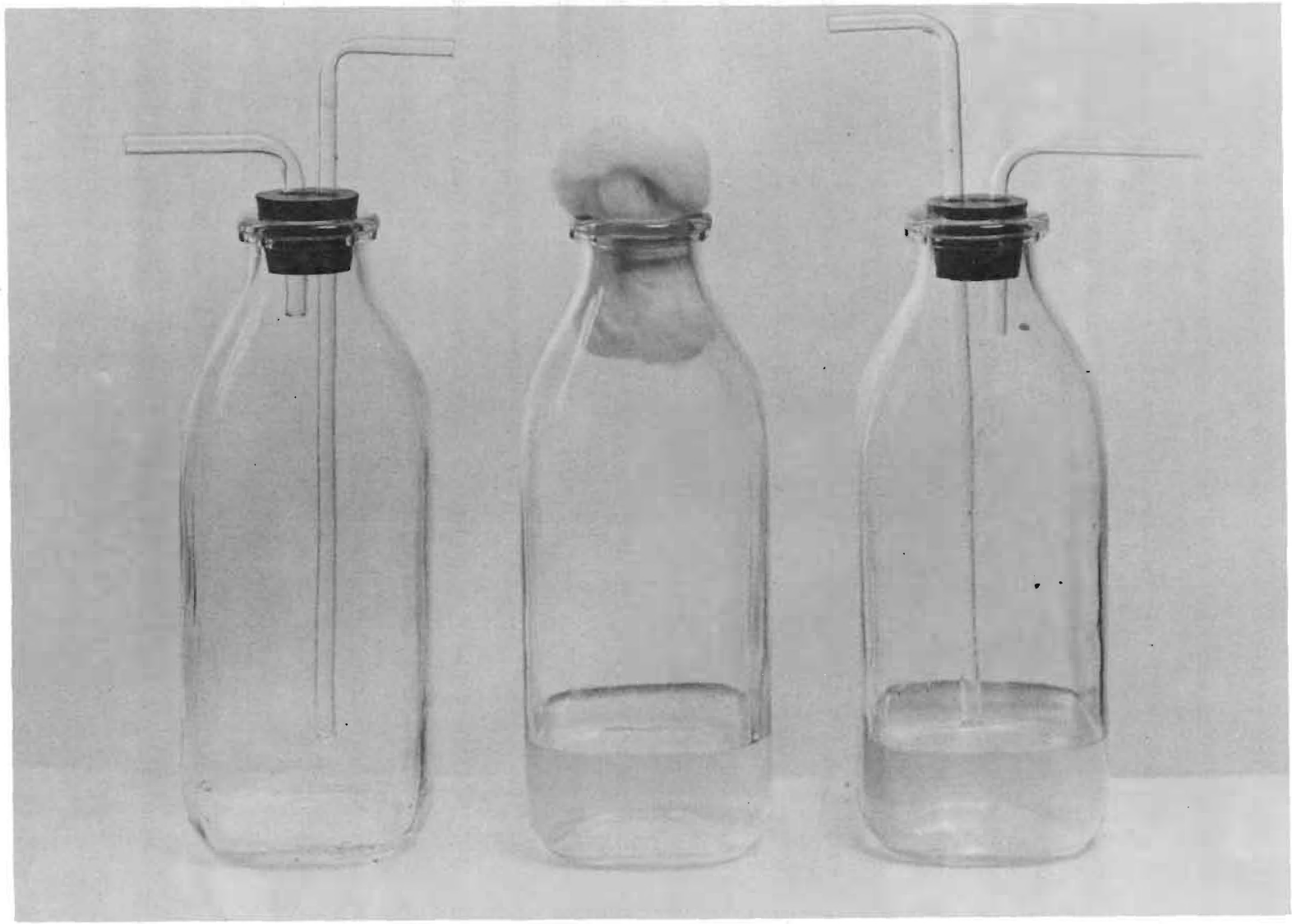


Figure 7. Critical-Orifice Liquid Impingers.

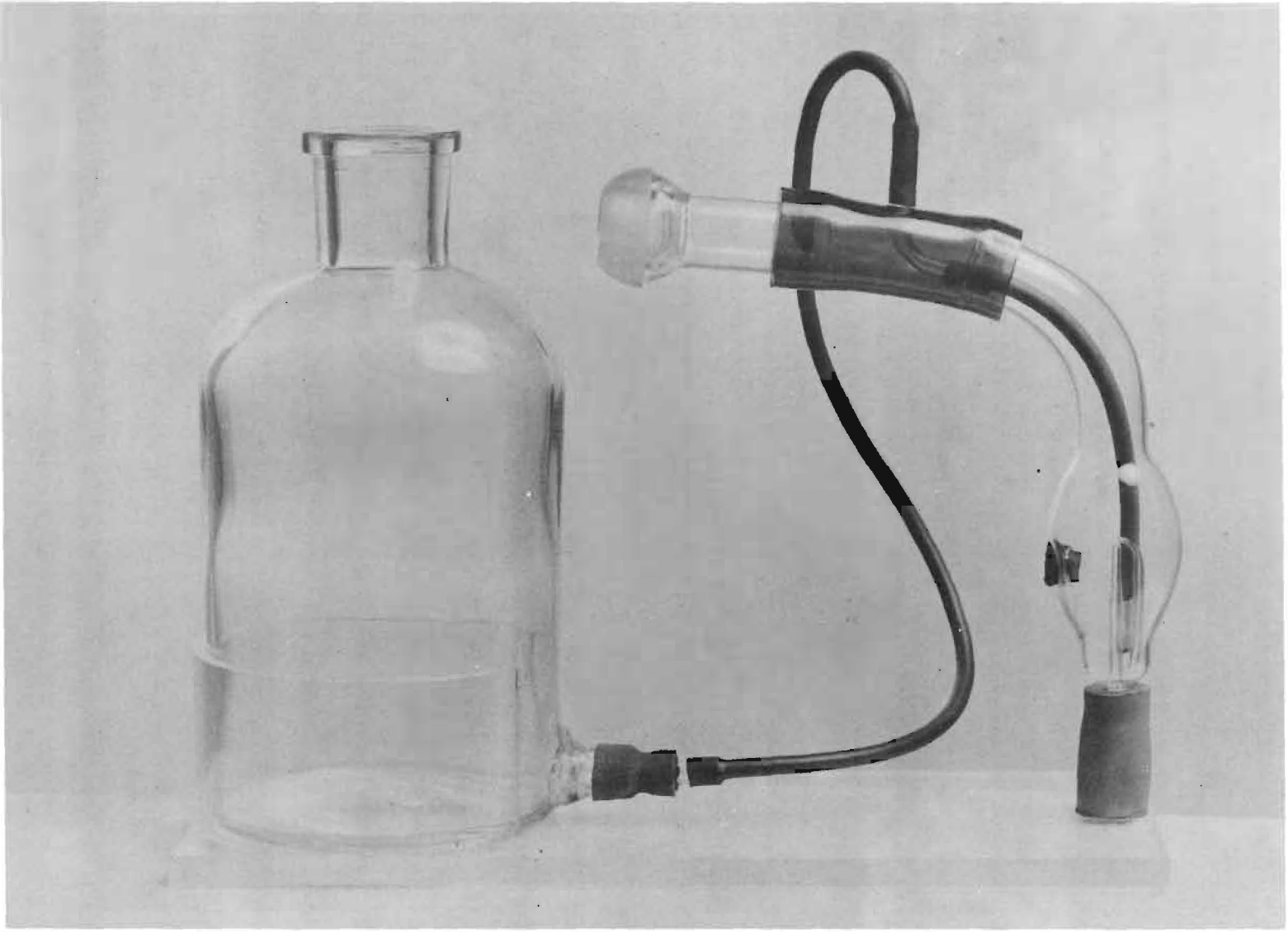


Figure 8. Reservoir and Atomizer.

system, or other uses, and has nothing to do with the atomizer unit. The air supply to the atomizing system is treated rather extensively to insure adequate and uniform pressure, temperature, and humidity regulation. Air from the compressor which furnishes air for general services within the research building is filtered and reduced from approximately 125 to 60 psi with a Binks No. 83 A regulator (Binks Manufacturing Company) and then further reduced and controlled at 30 psi by a Cash special pressure-controlling valve (A. V. Cash Company). Then the air is passed through two oxygen-type cylinders, 35-liter capacity each, located in a refrigerated unit maintained at 40° F. This part of the system serves as a surge tank and regulates, to a certain extent, the humidity of the air. At the exit of the surge tank units, but also in the refrigerated unit to assure constant temperature, is located a more sensitive Cash pressure-regulating valve, which maintains the air at a very constant pressure of 20 psi. It is this air which issues from the valve marked "Atomizer Air" in Figure 6.

The aerosol chamber can be operated either dynamically or statically. In the latter case, the chamber is sealed off by closing the sliding valves located in the entrance and exit pipes of the chamber. Settling samples can be taken within the chamber during each type of operation. The settling sample containers are located beneath each of the 4-inch holes in the bottom of the chamber. The actual sample holders were machined 1-inch diestock. Each holder contains a shutter for sealing off the 4-inch hole in the floor of the chamber, a cavity for holding Petri dishes, and a door on the bottom which can be closed so as to be airtight. In operation all components are airtight under at least 1 inch of water differential, so that when the sliding shutter is closed

the chamber hole is sealed, a Petri dish can be inserted into the cavity of the holder, and the bottom door of the holder closed. Then the Petri dish can be exposed to the chamber by opening the sliding shutter. Thus, it is possible to take settling samples as desired without disturbing the conditions within the chamber. Each settling sample holder is equipped with a small air inlet and outlet, controlled by a three-way stopcock, in order to purge the cavity of the holder after the Petri dish has been exposed. The control handles which operate the settling port slide covers and the valve to the chamber exit are shown in Figure 9 with the center pull being that for the exit valve. The stopcocks for operating the purging system for the settling-plate holder cavities may be seen just below the slide pulls, and the manometer indicating proper balance of inlet-exit flow of the purge system is located on the projection near the top of the chamber side. In the background and to the right of the chamber is the filter and exhaust blower, or exit. The three sampling tubes in the duct leading from the exit blower to the outside of the workroom are used for checking the efficiency and condition of the filter unit.

The inlet blower for the chamber draws air from the workroom or from a cubic-meter chamber which is employed for setting up concentrations of chemical vapors to be studied in the chamber. The exit blower for the chamber discharges through a filter system and outside the workroom.

The aerosol cylinder system and chemical-vapor-generating unit is shown in detail in Figure 10. Flexible stand construction was used to allow for later changes and addition of a third cylinder, if desired. Though aerosol is run through only one cylinder unit at a time, two basic cylinder units are arranged so that the aerosol flow may be directed immediately from one to the

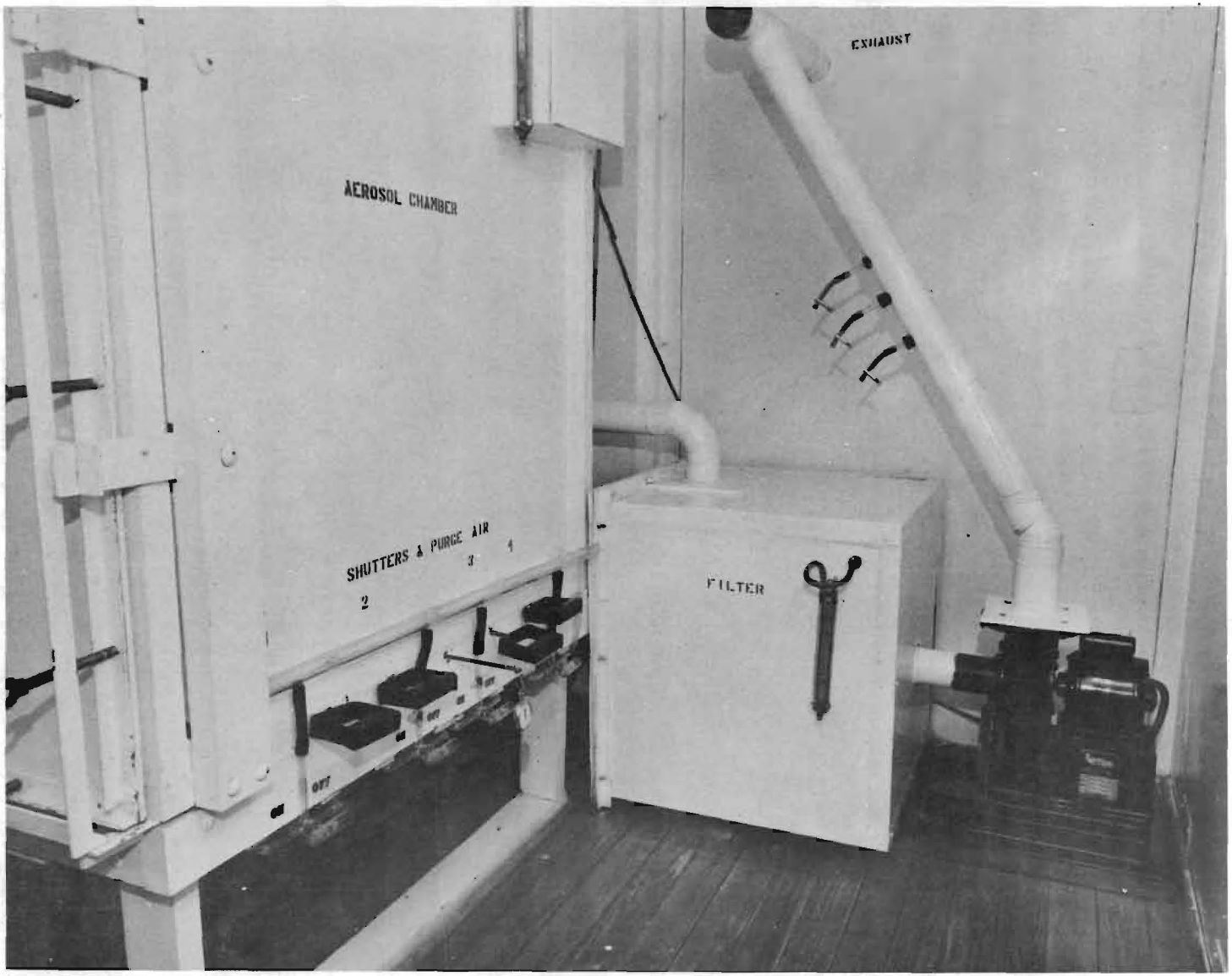


Figure 9. Side View of Aerosol Chamber.

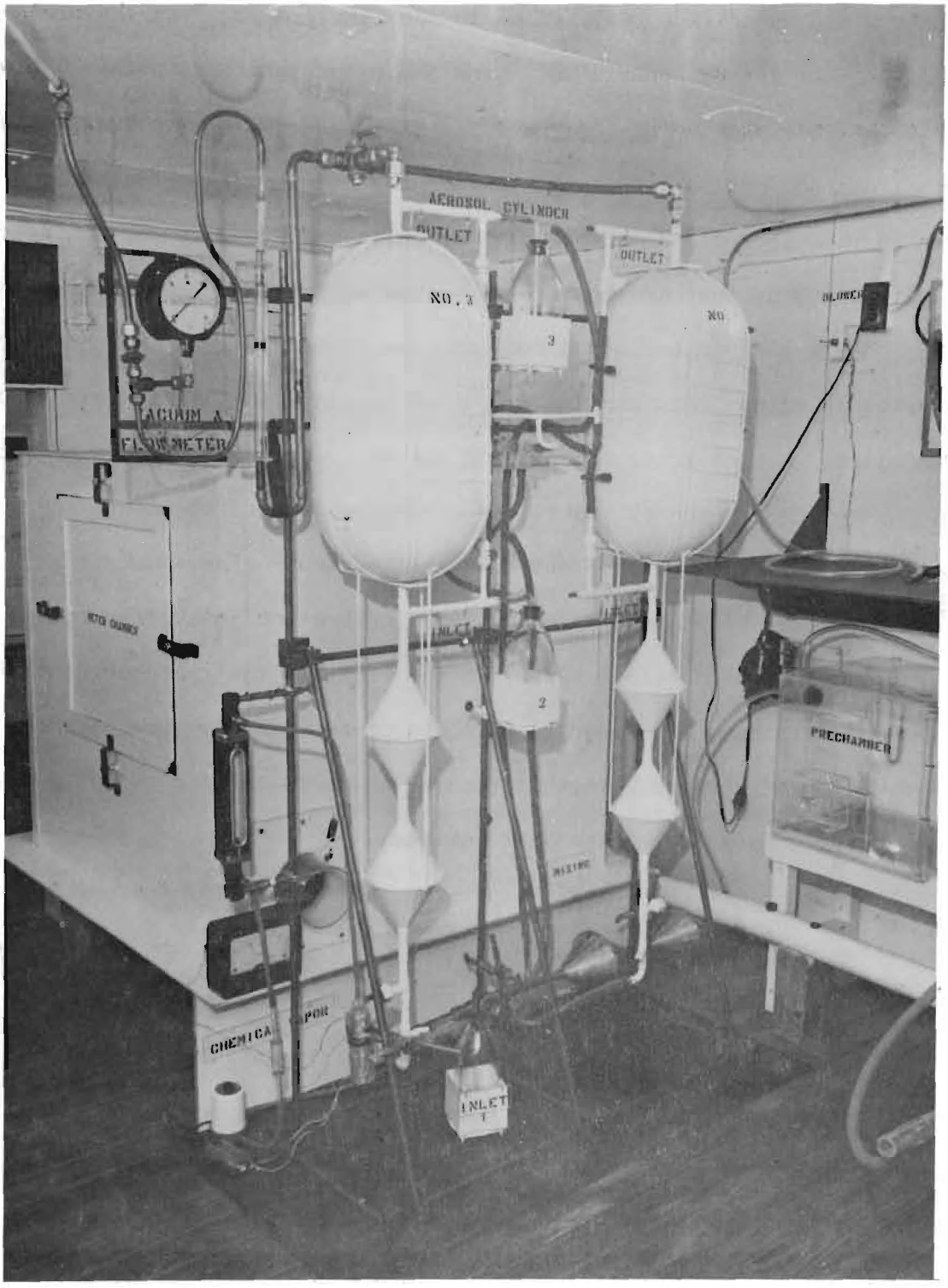


Figure 10. Aerosol Cylinders.

other. The source of the aerosol used in the cylinders is the same as that for the chamber, and an orifice restriction in the line leading from the prechamber to the horizontal mixing funnels maintains the same backpressure in the prechamber as is the case when using the chamber. Filtered room air is drawn into the horizontal mixing section along with the aerosol and is then directed by a valve into one or the other of the basic cylinder units. Where very low-humidity conditions are desired, the make-up air may be passed through a container of silica gel. The chemical vapor is introduced into the system just beyond the entrance of the aerosol and the main air and is pulled upward with them and mixed in the vertical mixing section prior to entering the cylinder. The mixture of vapor and aerosol passes through the cylinder, the flowmeter, the critical orifice which controls the flow rate, and then out through the vacuum line. Sampling tubes are provided at the inlet to the vertical mixing section just prior to the point of introduction of the chemical vapor, at the inlet to the cylinder, and at the outlet of the cylinder. In order to maintain pressure and volume balances in the system, a volume of air equal to that removed in sampling is constantly withdrawn from the system during the nonsampling periods.

The components of the chemical-vapor-generating unit for the aerosol cylinders are shown in Figure 11. In this figure, at the left is the small diaphragm pump (60 to 1200 cc/min) which forces air from the silica gel tube through the midget impinger containing the compound to be vaporized. Also on the left, an impinger is shown standing beside the heating unit containing another impinger. The temperature of the heating unit is adjusted by varying the voltage delivered to the heating unit until the desired temperature is indicated on the microammeter (shown in the center of Figure 11). This microammeter is connected to a copper-constantan thermocouple imbedded in the heating unit. The chemical-vapor generator used for setting up concentrations of compounds in the meter

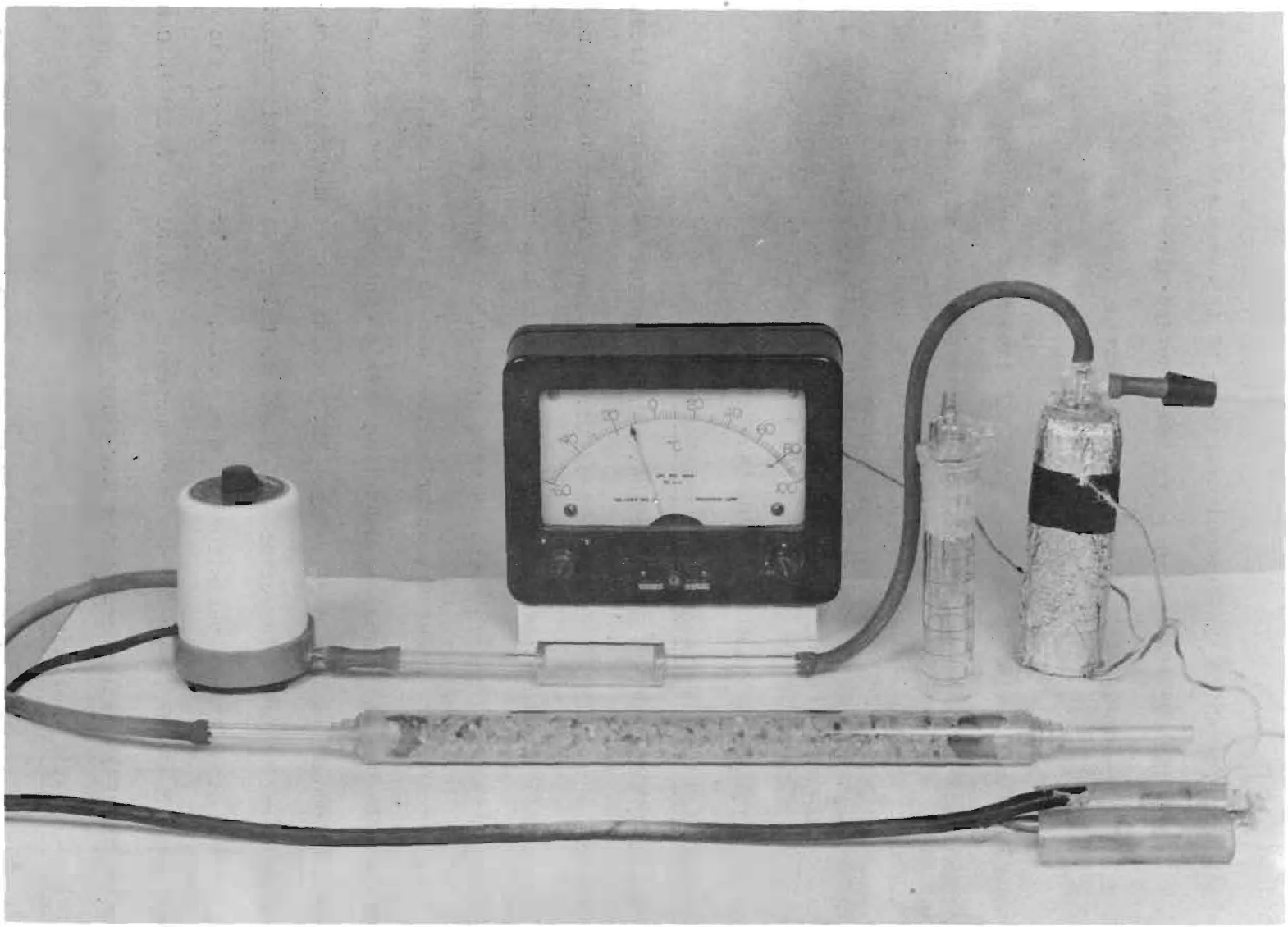


Figure 11. Small Chemical Vaporizer.

chamber prior to use in the aerosol chamber is simply a larger model of the unit shown in Figure 11.

The method of cleaning the aerosol cylinders is illustrated in Figure 12. Live steam is run through the cylinder, followed by dry air. The cylinder is then returned to the workroom for thermal equilibration prior to reuse.

Although the majority of the experimental work carried out on this project was done in the insulated workroom with the equipment described, the culture room and general laboratory facilities were essential adjuncts. A general view of the exterior of the workroom, balcony, and culture room is shown in Figure 13. A view of the transfer table within the culture room is given in Figure 14. A general view of the laboratory (separated from, but convenient to, the other facilities) is shown in Figure 15.

B. The Production of the Standard Bacterial Aerosol

A standard, reproducible bacterial aerosol is a prime necessity in studies of experimental aerobiology. The output of the prechamber system (described previously in this chapter) has constituted the standard bacterial aerosol in all the studies reported herein. The various precautions taken in the production of this aerosol were necessary in order to minimize two different types of variation; minute-by-minute changes occurring within each day's runs and variations possibly occurring from day-to-day. The combination of mechanical controls and cultural controls (described in detail in Chapter IV of this report) has maintained both of these variations at a minimum. A description of the individual particles making up the standard bacterial aerosol is found in Chapter V under "The Size and Nature of the Air-borne Particle."

1. Variations Within Runs

The operations involving the use of the aerosol cylinders for the screening of candidate aerial disinfectants necessitated the continuous operation

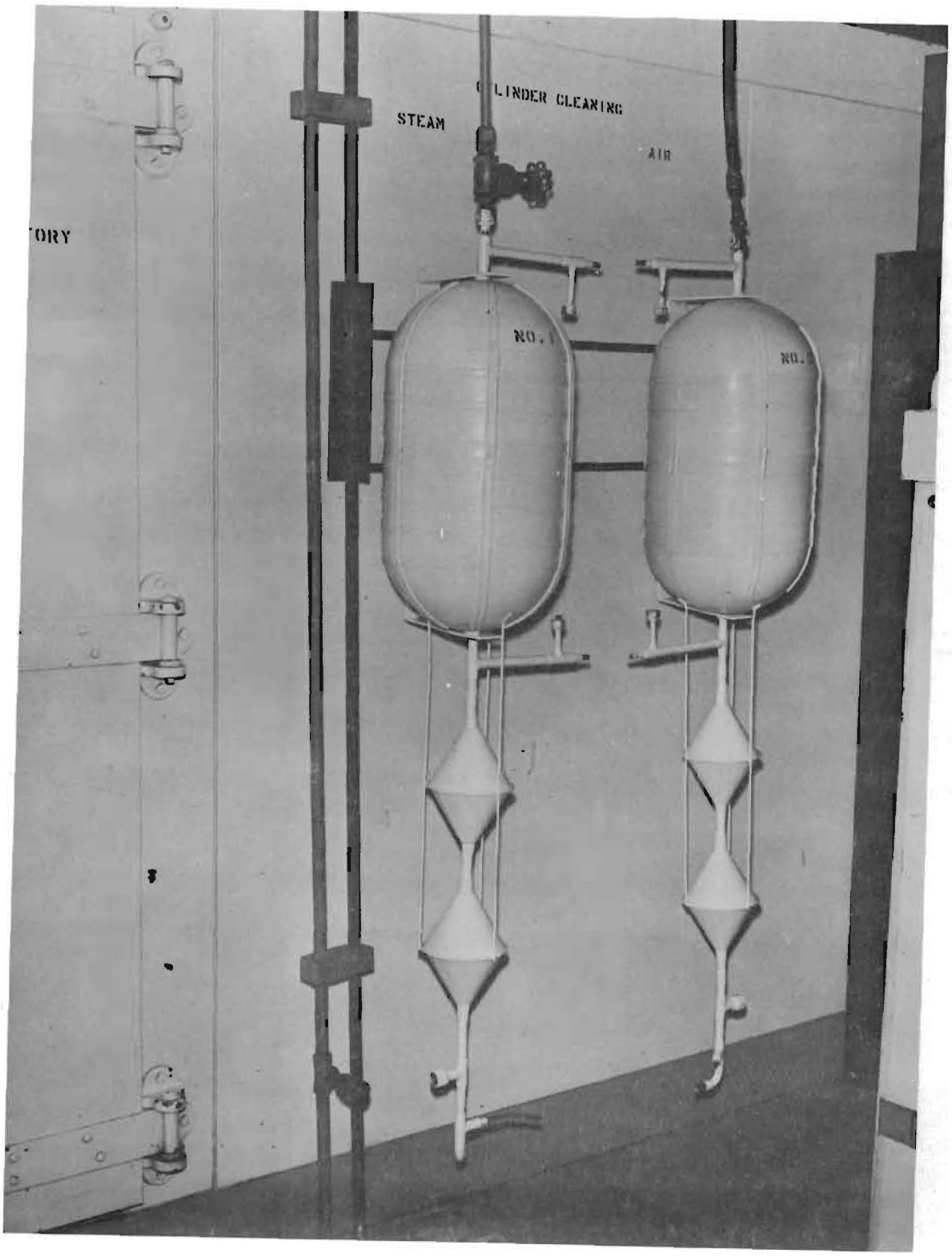


Figure 12. Cleaning Aerosol Cylinders.

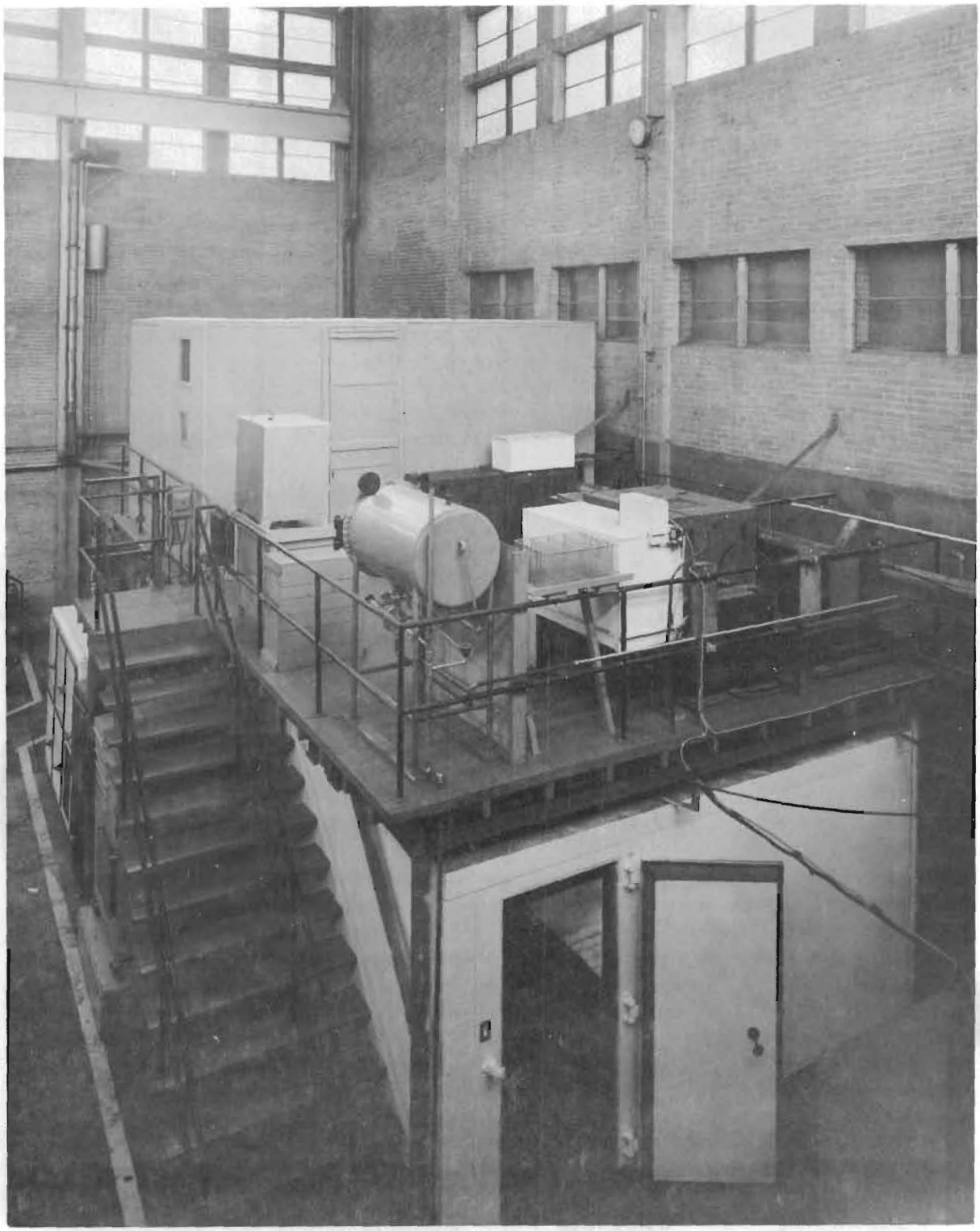


Figure 13. General View of Exterior of Workroom, Balcony, and Culture Room.

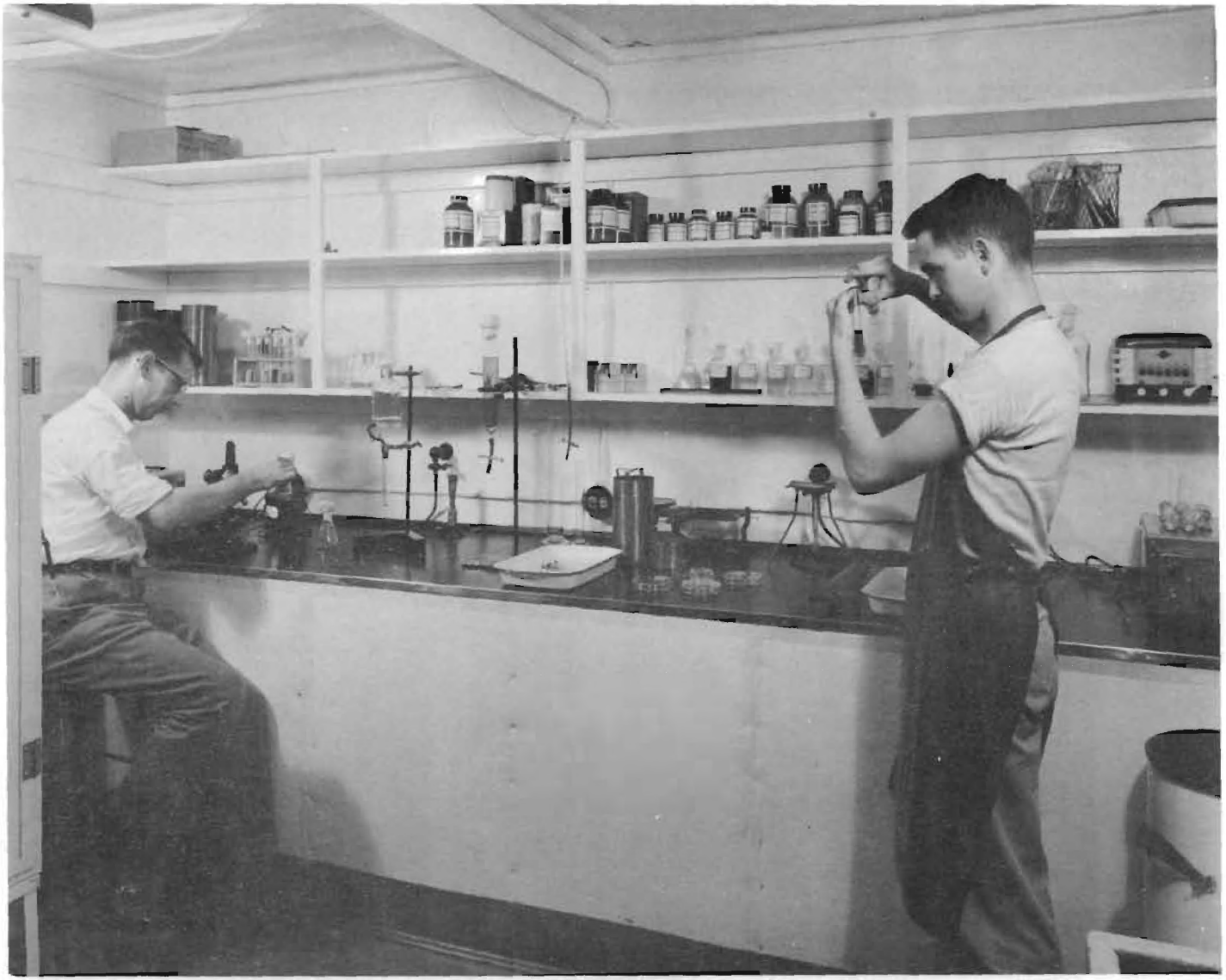


Figure 14. Transfer Table within the Culture Room.

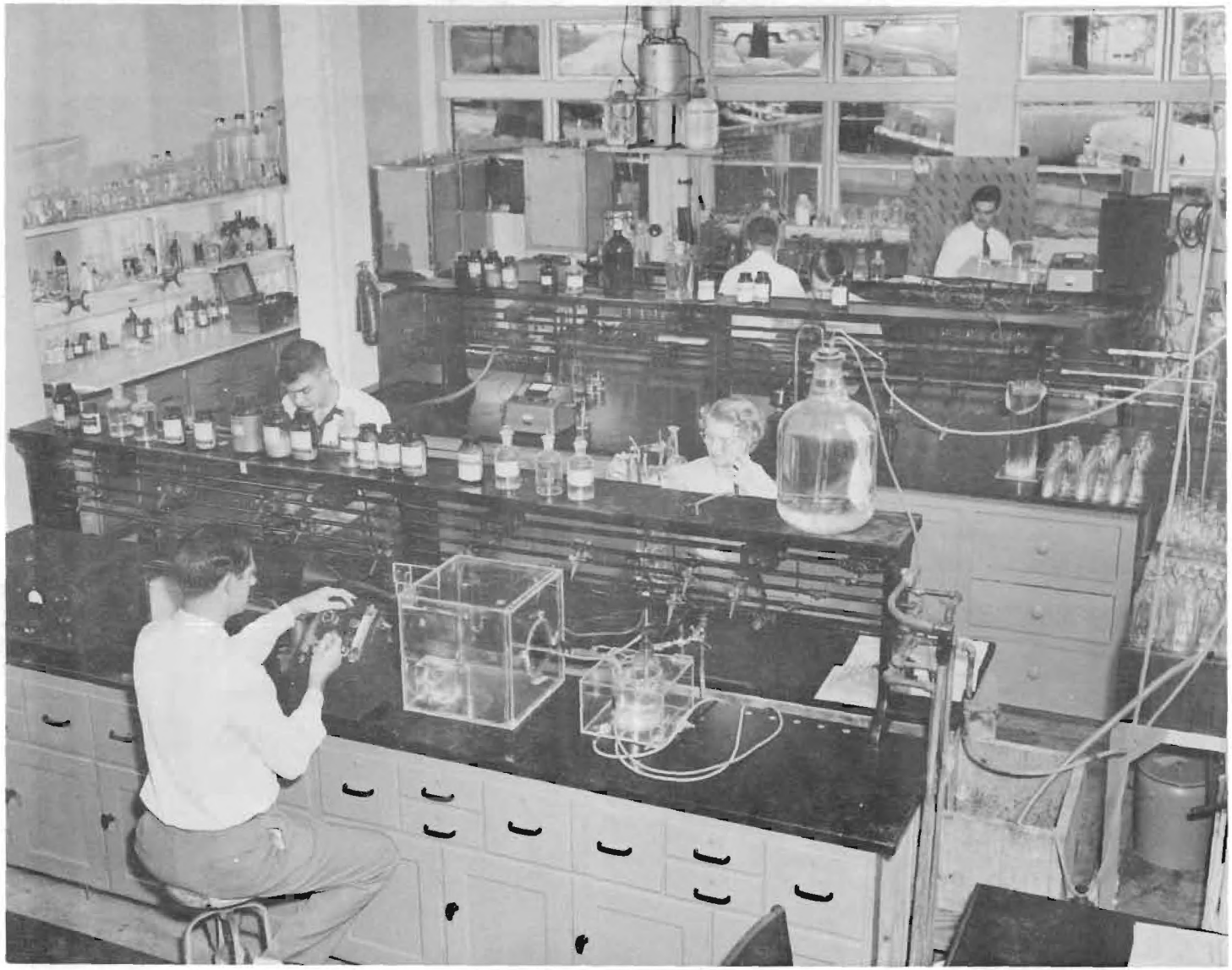


Figure 15. View of the Laboratory.

of the prechamber system for a period of not less than 6 hours each day. Although past experience had shown that the output of the prechamber remained essentially constant during such an operation, a very exact control of the aerial concentration of the bacterial aerosol was desired for the present work, and further investigations were made. It had been established that regulation of the atomizer air supply, control of the temperature of the atomizer bath, and control of the temperature of the room were all required for reasonably constant output by the prechamber. Under these operating conditions, a series of samples were taken at 15-minute intervals during the day. The resultant data indicated a constant output until the middle of the afternoon, followed by a definite increase in concentration of air-borne organisms.

This midafternoon increase in concentration occurred regularly and was finally traced to the operation of a sandblasting machine which drew large volumes of compressed air from the common compressor system. A pressure recorder was installed in the air line, and it was found that the operation of the sandblaster, usually just after lunch, caused an initial drop in the pressure, followed by an average increase, due to the continuous operation of the compressor. The system of reducer valves described in the preceding portion of this section was installed to reduce the variations in pressure to a minimum.

After the installation of the multiple reducers in the atomizer air line, the runs were repeated. It was found that the concentration now showed a definite tendency to rise regularly and gradually during a period of several hours. This difficulty was finally traced to a gradual buildup of numbers of organisms in the atomizer. Apparently, the cyclic fluctuation of pressure in the air lines had, in the past, caused sufficient variations in the flow through the atomizer

so that a continuous mixing of the contents of the atomizer and reservoir was effected. Eliminating the pressure fluctuations had permitted the buildup of an increased concentration of organisms in the atomizer.

In order to eliminate the increased concentration of organisms in the atomizer, the siphon pump was installed to effect continual mixing of the contents of the atomizer with those of the reservoir. The test runs were then repeated, and a vastly improved situation was found.

Typical runs from the above-mentioned series of tests are shown in Table I. In this table, "Condition 1" refers to the original operating conditions; "Condition 2" refers to the operating conditions after the installation of the multiple regulators in that atomizer air line; and "Condition 3" refers to the final arrangement with multiple regulators and the siphon pump. Operating under these various conditions, the last named condition yielded the most consistent concentration of air-borne organisms during the several hours of operation. All the data in Table I are derived from the results of samples of 1-minute duration; such variations as are found in this table under "Condition 3" are probably due to the shortness of the sampling period and the resultant probability of small errors in timing.

In the course of these studies, counts of total and viable organisms were made from the atomizer and from the reservoir prior to the runs and at the end of the runs. The beneficial effect of the siphon pump in effecting mixing between the atomizer and the reservoir is readily apparent when such data, as shown in Table II, are examined. Without the pump the concentration of cells, both total and viable, in the atomizer increased markedly. This concentration effect was completely overcome by the mixing action of the pump.

TABLE I

VARIATION IN NUMBERS OF VIABLE AIR-BORNE ORGANISMS ISSUING FROM PRECHAMBER DURING OPERATION AT 68° F, 60 PER CENT RELATIVE HUMIDITY

Time	Average Number of Viable Organisms Per Liter of Air x 10^{-4}		
	Condition 1	Condition 2	Condition 3
10:30 AM	---	252	---
45	---	208	---
11:00	---	249	264
15	286	300	260
30	288	239	340
45	253	294	314
12:00 Noon	258	349	294
15	250	410	284
30	250	409	225
45	257	398	330
1:00	168	492	300
15	266	445	265
30	216	535	284
3:00	455	510	---
15	450	605	---
30	470	593	270
45	483	690	320
4:00	565	415	286
15	545	555	280

2. Variation from Day-to-Day

In examining the output of the prechamber for variations which might occur from day-to-day, the most readily available data were those obtained from samples taken at the inlet to the aerosol cylinders. A large number of such samples were taken over a period of several months, all operations being identical except that the relative humidity was held at various conditions during these runs. The results of the samples taken following equilibration with the desired relative humidity conditions were accumulated for these various conditions. All numbers of viable air-borne organisms were calculated to an original culture count of 100 x 10^7/ml and were expressed in terms of concentration of viable organisms per liter of air issuing from the prechamber. The resultant averages, their respective standard errors, the number of individual observations, and the coefficients of variation are shown in Table III.

Examination of the information included in Table III indicates that there are some significant differences among the average number of viable organisms for several of the conditions of relative humidity. Subsequent analysis of this factor showed that this is probably due to a varying ability of the organisms to survive the change from near saturation in the prechamber to the particular relative humidity condition of the run. This varying ability to survive equilibration to rapid change in relative humidity is probably caused by the response to varying rates of drying. This is discussed in detail in Chapter V of this report.

The data in Table III show that, for any particular relative humidity, the coefficients of variation are reasonably small, including as they do all of the variations produced in operation, sampling, diluting, plating, and counting.

TABLE II

EFFECT OF SIPHON PUMP ON NUMBERS OF
ORGANISMS IN ATOMIZER FOLLOWING SIX HOURS OF OPERATION

Operating Conditions	Number of Organisms per Milliliter of Fluid x 10^{-7}					
	Initial Culture		Atomizer At End of Run		Culture Reservoir At End of Run	
	Total†	Viable	Total†	Viable	Total†	Viable
Pump Off	190	97	348	152	204	100
Pump On	200	86	200	83	212	82

† Total counts made by enumerating all cells in a Petroff-Hauser counting chamber

TABLE III

NUMBERS OF VIABLE AIR-BORNE ORGANISMS ISSUING FROM PRECHAMBER
AFTER EQUILIBRATION WITH ROOM AIR OF VARIOUS HUMIDITIES AT 68° F

Per Cent Relative Humidity	Average Number† of Viable Organisms per Liter of Air x 10^{-4}	Standard Error of the Average	Number of Observations	Coefficient of Variation
20-25	378	11.5	43	20.0
26-34	296	11.2	11	12.5
45-55	325	9.4	97	28.0
58-62	282	7.1	9	7.5
64-66	232	14.0	12	21.0
73-77	252	12.3	17	20.0
78-82	274	8.9	69	27.0
88-92	245	7.4	6	7.5

† Corrected to an original culture count of 100 x 10^{7}/ml.

That is to say, these data show, that at any time, it is possible to produce essentially the same bacterial aerosol in approximately the same concentrations, over a period of several months. Such a situation increases confidence in results obtained during such an interval, as well as simplifies the routine bacteriological procedures involved in diluting samples for counting.

C. Characteristics of the Main Aerosol Chamber

The main aerosol chamber, previously described, was designed in order to provide a device for studying, in detail, the behavior of bacterial aerosols. The requirements for such a device were given in an earlier report (Progress Report E-141-C) and are as follows: the chamber should be large enough to permit sampling without seriously disturbing its contents, yet small enough to be housed in most laboratories; the chamber and its environment should have adequate temperature and humidity controls; the chamber should operate equally well either statically or dynamically; provision should be made for obtaining both settling and air samples; in operation, its characteristics should be reproducible; the bacterial aerosol should be evenly distributed throughout the chamber; and, in operation, it should be possible to account for all the bacteria dispersed into the chamber. The present chamber embodies all of these characteristics.

1. Aerosol Distribution Within the Main Chamber

A study was undertaken to determine the distribution of the bacterial aerosol particles within the 4-foot-cube main aerosol chamber operated under dynamic condition of 60 cfm of air. The design estimate of the chamber equipped with a diffusing head for the incoming air-aerosol mixture indicated that a high degree of dispersion and homogeneous distribution throughout all points within the chamber could be expected. To determine the distribution of the bacterial

aerosol within the chamber, a vertical series of five apertures was made in the center of one wall of the chamber, and a similar vertical series of five apertures was made in one quadrant of the same wall. Reference to Figure 5 will show the arrangement of the apertures and their numbered designations. Straight glass-tube probes, 0.25-inch internal diameter, were inserted into these apertures, through rubber stoppers, to hold them firmly in place. The probe intruded into the chamber approximately 4 inches and was separated from the vertically adjacent probes by an interval of approximately 7.5 inches. This was the standard physical arrangement of the sampling probes. In the sampling of any of the aerosol clouds to determine the distribution of the cloud, various lengths and positioning of probes were used and have been so designated in reporting the results. Unless otherwise specified, all probes are in the standard position.

References to Tables IV and V show the results of concomitant samples taken from various points within the chamber during dynamic operation. The positions of the probe are such as to allow for adequate sampling of the interior of the chamber, as well as the surface of the chamber wall and the region of wall-floor junction. The total volume of aerosol sampled is approximately the same in each experiment, with the notable exception of the 5-liter-per-minute samplers exposed for 35 minutes. These data reflect a very good distribution of the bacterial aerosol throughout all portions of the chamber, and the variations encountered are within acceptable limits of error.

Preliminary studies of aerosol clouds composed of various smokes or bacteria demonstrated the characteristic of a cloud to maintain an integrated form resisting dispersion and dilution when introduced into a chamber. Visualization of the cloud by a Tyndal beam showed that the pattern of movement is imparted

TABLE IV

AEROSOL DISTRIBUTION WITHIN THE MAIN AEROSOL CHAMBER[†]

Probe No.	Sampler Capacity (l./min)	Concentration of Bacteria Per Liter of Air[††] Experiment No.1 Run 1	Experiment No.1 Run 2	Experiment No. 2 Run 1	Experiment No. 2 Run 2
1[†††]	5	2780	3100	2760	2080
2	1	3000	3500	2610	2210
4	1	3130	2840	2710	2440
5[††††]	5	2780	4000	2120	1875
8	1	3280	4550	3100	----
8	2	----	----	----	3420
	Average =	2994	3598	2660	2405
	σ =	219	689	354	601
	C.V. =	7.3	19.2	13.3	25

† Culture: S. marcescens, ATCC 274, E-R
Atmosphere: dry bulb 76° F, relative humidity 70 per cent
Sampling Period: 35 minutes
Sampling Medium: 0.2 per cent gelatin solution, buffered

†† Concentration of bacteria per liter of air not corrected to 100×10^7/ml original culture.

††† Orifice of sampling tube contiguous to chamber wall.

†††† Orifice of sampling tube contiguous to chamber floor.

by the nature of the introducing orifice, and, in a condition of nonturbulent flow, the entering cloud may be followed for a considerable period of time within the chamber. Devices of various designs for disrupting the cloud by producing turbulent mixing were interposed in the entering conduit, and/or within the

TABLE V

AEROSOL DISTRIBUTION WITHIN THE MAIN AEROSOL CHAMBER†

Location of Sampling Probe	Probe No.	Run 1 (conc/l.)	Run 2 (conc/l.)	Probe No.	Run 1 (conc/l.)
2 feet from wall	1	2890	2640	6	1750
1 foot from wall	2	2420	2860	7	2720
2 inches from wall	3	2960	2810	8	2700
2 inches from wall and floor	4	2700	3000	9	1960
2 feet from wall	5	2570	2900	10	2580
Average =		2708	2842		2342
σ =		223	133		454
C.V. =		8.2	4.7		19.4

† Capacity of Impinger Sampler: 5 liters per minute.

Sampling Period: 5 minutes.

Sampling Medium: 1.6 per cent brain-heart infusion - 0.2 per cent gelatin solution, buffered

Culture: S. marcescens, ATCC 274, E-R.

Atmosphere: Dry bulb 40° F, relative humidity 57 per cent.

chamber. The failure to disperse the cloud amply demonstrated the resistance of the cloud to any dilution effects. Stirring apparatus within the chamber would be undesirable because it would not provide the random distribution under dynamic conditions which are necessary but would produce flow patterns of palpable velocity. The qualities of the observed cloud present a fundamental aspect of chamber design requirements to be met by any chamber system providing satisfactory conditions of random samples during dynamic or static operating conditions.

The present design of the main aerosol chamber incorporating the Anemostat diffusion head has resulted in a chamber which provides a continuously operating system, producing a homogeneous dispersion of a bacterial aerosol cloud. During months of operation, the system has shown a high degree of reliability, and it has provided the basic component in the study of microorganisms, sampling devices, and related techniques of experimentation.

2. Dynamic Filling and Emptying Characteristics of the Main Chamber

The inimical effect of different atmospheres on air-borne bacteria is expressed as k in this report and is obtained from the relationship of the log of the initial number of organisms (N_o) minus the log of the final concentration of organisms (N_1) divided by the time (t) of exposure. In the operation of the main aerosol chamber, a 4-foot cube, the factor t is equal to the time required for the passage of 99 per cent of a bacterial aerosol cloud through the chamber, and it is determined by the rate of flow of the aerosol. In the dynamic operation of the main aerosol chamber, the rate of flow has been 60 cfm. To determine the value t for the chamber operated at 60 cfm, an aerosol of S. marcescens ATCC 274, E-R, was introduced into the chamber for not less than 15 minutes to insure complete equilibration before brain-heart-gelatin, critical-orifice, impinger samplers were employed to determine the bacterial concentration. At time t, the bacterial aerosol was by-passed from the chamber, and impinger samplers of 1-minute exposure were started. As the chamber was emptied, samples were taken in 1-minute sequences, $t_1 \ldots t_5$. All samples were taken from the chamber exit.

The results are shown in Table VI and indicate that the time required for the passage of 99 per cent of the aerosol cloud through the chamber, under the dynamic conditions stated, is 5 minutes. To compare the experimental data with

TABLE VI

DYNAMIC EMPTYING CHARACTERISTICS OF THE MAIN AEROSOL CHAMBER

Per cent† Residual Aerosol	Time (min)	Bacterial Concentration Per Liter of Air			
		Run 1		Run 2	
		Actual	Theoretical	Actual	Theoretical
100	0	2695	2695	6470	6470
39	1	3021	1050	5250	2520
15	2	1265	403	1650	973
6	3	485	161	860	389
2.4	4	167	64	260	155
1.5	4.5	----	40	----	97
0.9	5	80	13	90	58

† Per cent residual aerosol = $100\ (e^{-\frac{f}{v}t})$
where f = chamber air-flow rate = 60 cfm and
v = chamber volume = 64 ft^3.

the expected theoretical disappearance of the aerosol, the formula expressing the per cent residual aerosol at any time t is used. This formula is

$$\text{per cent residual aerosol} = 100\ (e^{-\frac{f}{v}t})$$

where f = chamber flow rate, cfm,

v = chamber volume, ft^3, and

t = time in minutes.

This formula is based on the ventilation characteristics of a gas and presumes perfect and instantaneous mixing or diffusion. It will be noted that the experimental and theoretical results are in acceptable agreement relative to numerical

magnitude but are approximately 1 minute out of time phase, e.g., the corresponding experimental value is 1 minute later than the theoretical value. This result is derived from the fact that a bacterial aerosol cloud does not possess the comparable high rate of mixing and diffusion characteristics of a gas and from the fact that the impinger sample of t_1, started at the same time as the aerosol was by-passed, was actually sampling an undiluted aerosol cloud. This 1-minute lag of the experimentally derived emptying characteristic of the chamber does not invalidate the determined value of 5 minutes for the time required for the passage of 99 per cent of an aerosol cloud through the chamber operated at 60 cfm.

The filling characteristics of the main aerosol chamber were similarly determined but in reverse order. Into the dynamically operating chamber, a bacterial aerosol was introduced and critical-orifice impinger samples were taken to monitor the increasing concentration of aerosol within the chamber. From the ventilation equation, it is apparent that the chamber concentration, at first, rises rapidly and then approaches a constant value at infinite time. The experimental data, Table VII, confirm this rapid change. The initial high rate of change (61 per cent) during the first minute of introduction into the chamber presents a considerable problem in the collection of a sufficient number of organisms during very brief intervals of time using the 1-liter-per-minute sampler. During the initial phase, samplers were operated for a single period of 3 minutes and, subsequently, for periods of 1 minute each. Although the initial phase of the filling characteristic was obscured to a degree, the first and subsequent samplers indicated that the chamber exhibited an acceptable performance in accordance with the expected filling characteristics.

TABLE VII

DYNAMIC FILLING CHARACTERISTICS OF THE MAIN AEROSOL CHAMBER

Theoretical Performance			Experimental	
Per Cent† Aerosol	Time (min)	Air (conc/l.)	Time (min)	Air (conc/l.)
0	0	0	0	0
61	1	3840	0-3	3240
85	2	5350	3-4	4650
94	3	5930	4-5	5700
97.6	4	6150	5-6	6450
98.5	5	6200	6-7	6450
99.1	6	6300††		

† Per cent aerosol = $100 (1 - e^{-\frac{f}{v} t})$
where f = chamber flow rate = 60 cfm,
v = chamber volume = 64 ft^3, and
t = time in minutes.

†† Average concentration of bacteria per liter of air after approximately 10 minutes of chamber equilibration.

In order to determine whether or not the initial lag exhibited during the emptying of the chamber is characteristic of the aerosol cloud or of the chamber itself, data were obtained on the filling characteristic of the chamber using water vapor. From the mathematical expression of theoretical emptying and filling characteristics, it is evident that determined values are convertible. Water vapor, as a gas, should diffuse instantly, and any significant variation from the theoretical results would indicate that the chamber is not operating properly. In this experiment, water vapor was introduced into the chamber by

atomization at such a rate as to give a final concentration equivalent to about 80 per cent relative humidity. The room air was maintained at about 20 per cent relative humidity. Under these conditions, the output of the thermal conductivity cell could be set at zero for the ventilation of room air through the chamber, and, after prolonged atomization of the water into the chamber, an output signal of about 5.0 mv (one-half full scale) could be obtained. These conditions of operation were chosen to obtain maximum sensitivity of the thermal conductivity cell without ever approaching a limiting value of saturation. The thermal conductivity cell output is directly proportional to the amount of water present in the air.

Several runs were performed under the conditions outlined above, and agreement was obtained among the resulting curves plotted on the Esterline-Angus recorder. These data are shown in Table VIII, as are the theoretical values for per cent equilibrium. In general, there is reasonable agreement between the actual and the theoretical values. It is concluded that the 99 per cent equilibration time for the chamber is as calculated and is in agreement with the data obtained from the study of the emptying characteristics of the chamber employing bacterial aerosols.

In summary, the performance of the dynamically operated aerosol chamber has demonstrated the soundness of design in providing an apparatus for the production and containment of a homogeneous bacterial aerosol cloud. The reliability of production and dissipation as well as the dispersion of the aerosol cloud within the chamber provides a primary basis of confidence in the subsequent studies on air-borne bacteria.

TABLE VIII

DYNAMIC FILLING CHARACTERISTICS OF THE MAIN AEROSOL CHAMBER USING WATER VAPOR AS INDICATOR

Time (min)	Thermal Conductivity Output† (mv)	Per Cent Equilibration	
		Actual	Theoretical††
0.0	0.0	0.0	0.0
0.625	2.7	49.0	44.6
1.25	4.0	73.0	69.0
1.875	4.5	82.0	84.7
2.5	5.0	91.0	90.4
3.75	5.3	96.5	97.1
5.0	5.5	100.0	99.1
6.25	5.5	100.0	99.7

† The thermal conductivity cell output was adjusted to zero prior to the introduction of water vapor into the chamber.

†† Per cent equilibration = $100 (1 - e^{-\frac{f}{v} t})$
where f = chamber flow rate = 60 cfm,
v = chamber volume = 64 ft^3, and
t = time in minutes.

D. Characteristics of the Aerosol Cylinders

Although the main aerosol chamber has proven to be an excellent chamber component in the system for the production and containment of bacterial aerosol clouds under continuously operating conditions, there are inherent disadvantages in its use as an apparatus for the screening of chemical compounds for aerial disinfection. This disadvantage is primarily one of chamber size and operational

detail. Following the establishment of a standard bacterial aerosol cloud and its subsequent treatment with a chemical compound, it is necessary to isolate the chamber component for meticulous cleaning of all surfaces in order to remove traces of the chemical tested. Although this is quite feasible with the chamber proper, such a procedure is time consuming, and, furthermore, the permanent installation of conduits presents a problem. However, where the detailed study of previously selected and tested chemical compounds is desired, this procedure is not excessive for the aims and value of such detailed study.

The above requirements indicated the need for a much smaller chamber, one easily manipulated and having the same general characteristics of the aerosol chamber. In general, these characteristics are: uniformity of distribution of the bacterial aerosol throughout the system; a measurable detention time; capability of operating at various temperatures and relative humidities; and size--large enough that the sampling operation does not produce dilution errors. The low-pressure, nonshatterable, oxygen cylinder used by aviators during high-altitude flying was found to meet the requirements of such a chamber almost exactly. These cylinders are made of stainless steel, are 12 inches in diameter, having a straight cylindrical midsection 12 inches in length, and are 24 inches in overall length, the ends being dome-shaped. The volume of the cylinder is about 35 liters. The cylinders may be used separately or paired in series, depending upon the detention time desired. The single- or dual-cylinder system is operated at 30-liters-per-minute air flow, a flow which approaches the capacity of the available vacuum pumps when operating across an orifice at the critical-pressure ratio. This method of exactly controlling the flow is a most satisfactory one and is to be preferred whenever practical. The system is operated

with an upward air flow, and the 30-liters-per-minute flow provides sufficient upward velocity to maintain the aerosol particles air-borne at all times.

The determination of the aerosol detention time for the cylinders was necessary because the k factor for the cylinders is determined in the same manner as for the aerosol chamber, e.g., $\log N_o - \log N_1$ divided by the detention time. The emptying characteristics for the dual-cylinder system--a system of two cylinders connected in series--were determined and are reported in Table IX. The general characteristics of the dual-cylinder system appear to agree with the theoretical considerations of a chamber of these dimensions. In the time sequences later than 5 minutes, the values obtained differ from the theoretical values, the actual emptying rate of the cylinders showing an increase. This increased rate of emptying may be caused by a parabolic profile of the withdrawing aerosol cloud which is exhibiting terminally a more rapid dilution and removal. Also, it is to be recalled that the cylinders are arranged in series and, although considered as a single chamber, the system may possess some characteristics peculiar to this arrangement. Several measurements have indicated an emptying time of slightly over 7 minutes, and, for ease of computation, 7 minutes was accepted as the aerosol detention time for the dual-cylinder system.

The routine, chemical-screening tests were conducted employing only one cylinder at a time. The detention time was estimated to be approximately one-half of the dual-cylinder system's time and, to verify this, the dynamic filling characteristics of the single-cylinder system was determined directly using a bacterial aerosol cloud and, also, water vapor. Since the essential factors of the ventilation formula are common to both the filling and emptying phenomena, the data for filling or emptying are directly comparable.

TABLE IX

DYNAMIC EMPTYING CHARACTERISTICS OF THE DUAL CYLINDER

Per Cent† Residual Aerosol	Time (min)	Bacterial Concentration Per Liter of Air			
		Run 1		Run 2	
		Actual	Theoretical	Actual	Theoretical
100	0	-----	50300††	-----	57300††
65	1	33200	33200	37800	37200
42	2	25700	21100	33700	24000
27	3	14300	13600	16000	15450
18	4	8700	9050	9800	10300
12	5	5000	6030	4750	6850
7	6	2340	3520	2930	4000
5	7	1500	2510	1420	2860
3	8	670	1510	700	1715

† Per cent residual aerosol = $100\ (e^{-\frac{f}{v}t})$
where f = chamber air-flow rate = 30 liters per minute, and
v = chamber volume = 68.8 liters.

†† Values derived from experimental values at 1 minute.

In determining the dynamic filling characteristics of the single-cylinder system, water vapor was introduced into the inlet, and the rise in concentration of water vapor at the outlet was recorded directly from the response of a thermal conductivity cell located at the outlet. The data resulting from the use of water vapor as an indicator, as reported in Table X, appear to be in agreement with the theoretical values. The data also shows a tendency of increased rate of equilibrium as a function of time, as was seen in the emptying characteristics of

TABLE X

DYNAMIC FILLING CHARACTERISTICS OF THE
SINGLE CYLINDER USING WATER VAPOR AS INDICATOR

Time (min)	Thermal Conductivity Output† (mv)	Per Cent Equilibration†† Actual	Theoretical
0.0	0.0	0.0	0.0
0.25	0.80	21.0	20.0
0.5	1.55	40.0	35.0
1.0	2.65	68.5	58.0
1.5	3.3	85.5	73.0
2.5	3.75	95.5	89.0
4.0	3.8	100.0	97.0

† The thermal conductivity cell output was adjusted to zero prior to the introduction of water vapor into the cylinder.

†† Per cent equilibration = $100\ (1 - e^{-\frac{f}{v}t})$
where $\underline{f}$ = chamber flow = 30 liters per minute, and
$\underline{v}$ = chamber volume = 34.4 liters.

the dual-cylinder system. The possible explanation given previously for this behavior is applicable to the filling characteristics. The results obtained when using a bacterial aerosol cloud as an indicator, as reported in Table XI, are not as helpful because of the exponential increment of the admitted cloud-air mixture and the minimum practical time of the sampling method. The general results show a basis for accepting the detention time value of 3.5 minutes for the single-cylinder and 7 minutes for the dual-cylinder system.

The results obtained from prolonged usage of this apparatus indicate a high degree of reproducibility of operation. The objectives of the original design

TABLE XI

DYNAMIC FILLING CHARACTERISTICS OF THE SINGLE CYLINDER

Per Cent Aerosol†	Theoretical Performance		Experimental	
	Time (min)	Air†† (conc/l.)	Time (min)	Air†† (conc/l.)
0	0	0	0	0
58	1	121	0-1	42
83	2	173	2-3	123
93	3	194	4-5	195
95	4	198	6-7	206
97	5	204	---	---
99	-	209†††	---	---

† Per cent aerosol = $100\ (1 - e^{-\frac{f}{v}t})$
where f = chamber flow rate = 30 liters per minute, and
v = chamber volume = 34.4 liters.

†† Concentration of bacteria per liter of air = N x 10^3.

††† Concentration after approximately 15 minutes' equilibration.

have been attained in an apparatus which permits the screening of chemical compounds at a rate greatly in excess of that possible with the main aerosol chamber. Because the cylinder system is much smaller than the aerosol chamber, it has the objectionable characteristics of many small systems, such as sampling dilution effects during operation. The value of the cylinder system may be enhanced by making possible the study of the effects of the atmosphere on several microorganisms in rapid succession, much in the same manner as is used for rapid chemical screening. In this latter regard, advantage can be made of the variable detention time of 3.5 and 7 minutes provided by the single- and dual-cylinder system,

as different organisms may exhibit wide differences in response to the lethal effects of the atmosphere. Longer detention times could be achieved by using additional cylinders in series. These features, together with the facility of cleaning, make this system extremely valuable in the study of air-borne bacteria.

IV. BACTERIOLOGICAL METHODS AND CULTURES

In planning the program of work for this project, the primary test organism, S. marcescens, was selected on the basis of natural occurrence, nonpathogenicity, relatively simple nutritive requirements, and other characteristics which facilitated the use of this bacterium without extraordinary procedures and techniques. The dispersing and collecting media were also selected to simplify the preparation and to satisfy the nutritive requirements of the bacterium. The expenditure of extended effort on this phase of the project was not considered to be of prime import at this particular time.

Several developments strongly emphasizing the nature of the organism and composition of the dispersing, collecting, and plating media in relation to the survival of air-borne organisms under various atmospheric conditions, whether natural or artificial, are presented in this chapter, as well as in other chapters.

A more critical examination of the stock culture than originally was planned was essential. The existence of at least four colonial forms of the various strains of S. marcescens was well recognized, and it had generally been assumed that, if a variant showed no appreciable percentage of any other variant, the culture was stable. However, as it will be pointed out in more detail in later discussions, even slight morphological changes may cause variation in resistance when the bacteria are suspended in the air.

Also, the concept of a secondary environmental effect for air-borne bacteria places primary importance upon the composition of the immediate environment of the bacterial particle in determining the fate of the air-borne bacterium. Beef-extract broth was selected as the dispersing medium because it is a common cultural medium and produces an excellent growth. Though other media might possibly

be more suitable, the primary emphasis of the study was not considered to be the selection of optimum media but rather the selection of suitable and useful media. The original concept of the state of the air-borne organisms held by the personnel of this project was that the single organism in an air-borne particle (as in these studies) is covered by only a thin layer of material derived from the solids of the substrate from which it is atomized. However, evidence that the air-borne bacterium is surrounded by a relatively thick layer of nonliving residual substrate material which forms the actual immediate environment of the bacterium indicates that the actual response of the organism may be obscured by effects peculiar to the presence of the accompanying material. This gives great importance to the nature of the substrate from which the organisms are atomized.

The same factors in selecting the dispersing medium also applied to the original selection of the collecting fluid used in the impinger samplers and the plating materials. Evidence obtained in instances where the organisms may have suffered varying degrees of injury shows that enriched media may be required to demonstrate these attenuated organisms.

The bacteriological methods and procedures used in the work covered by this report are presented in detail in the following sections.

A. General Methods

Standard bacteriological procedures were adhered to in the production of the bacterial test organisms. The bacterial specimens were obtained from the American Type Culture Collection. Transfers were then made into 0.3 per cent beef-extract broth, 60-ml volume, and incubated at 30° C. Serial transfers were subsequently made into beef-extract broth at regular intervals of 45 to 48 hours. Four or five transfers were made before using the culture in tests. Thereafter,

the culture was maintained in beef-extract broth and transferred at the time intervals stated. Stock cultures were maintained in nutrient agar butt tubes with a surface overlay of mineral oil by storing in the refrigerator (5° C). Subsequent culturing of the microorganism in beef-extract broth, according to the routine method described, demonstrated the stability of the culture as determined by $\underline{k}_t$ values under standard atmospheric conditions.

Studies of the growth curve of the organism in beef-extract broth at 22° C showed the maximum stationary phase to occur at approximately 40 hours. The age of the test organism used in the production of aerosol clouds was between 40 and 48 hours, and it was found to provide a physiologically and numerically constant culture for daily use.

All media were standard Difco preparations (Difco Laboratories). Glassware used in the bacteriological procedures was cleaned in a hot detergent (Institutional X, Proctor and Gamble) solution, rinsed in tap water, and finally rinsed in distilled water. All water used in making serial dilutions and in preparation of bacteriological media was first distilled and then passed through a mixed-bed, ion-exchange column (IRA 400 and IR 120, Rohm and Haas).

1. Plating Media

Enumeration of viable bacterial cells was made according to routine bacteriological procedure using solid media. During the early states of this work, nutrient agar was accepted as a generally standard medium suitable for the organisms being used. This medium produces countable colonies within 30 to 40 hours. As a result of testing several solid nutrient media, with the objective in mind of reducing the period of incubation of the bacteria before the counting of the colonies, a buffered, sodium chloride, tryptone-glucose-extract

agar medium was developed which reduced the necessary incubation time by one-half. The composition of the medium was tryptone-glucose-extract agar (24 grams), sodium chloride (5 grams), anhydrous dibasic sodium phosphate (2.5 grams), and deionized water (1 liter). Inoculated agar plates were incubated at the temperature of 35° to 37° C. This medium was adopted as the standard plating medium.

2. Plating Technique

Upon sampling the bacterial aerosol cloud, the impinger solution was plated directly and/or serially diluted in sterile deionized water for subsequent plating. Aliquots (direct and/or diluted) of the sample were plated in triplicate for determination of viable cell numbers. The culture medium (tryptone-glucose-extract agar, buffered as previously described) was maintained at 50° C in a water bath before being mixed with aliquots of the sample in Petri dishes of standard size (9 cm x 1 cm). After solidification of the agar, the plates were stored in an inverted position in an incubator at 35° C for approximately 18 hours. At the end of this time, colony growth was advanced sufficiently to permit enumeration with the Quebec colony counter.

B. Test Organism

The test organism used was S. marcescens (Bizio). This bacterium is of a genus naturally occurring in soil, water, and foods and is used frequently in aerosol studies. Bacterium size, nonpathogenicity, relatively simple nutritive requirements and chromogenicity are characteristics which facilitate the use of this bacterium without extraordinary procedures in technique.

A specimen of S. marcescens was obtained from the American Type Culture Collection, Culture No. ATCC 274. This culture was transferred into beef-extract broth and was maintained in broth by serial transfers at 48-hour intervals. For

the purpose of examining and verifying colony-type formation, dilutions of a 48-hour culture were plated into nutrient agar. Incubation was at 30° C for approximately 12 hours and then at 20° C for an additional 12 to 24 hours' growth. This procedure was found to give very good color development of the colonies. Typically circular, thin, smooth, orange-red colonies were selected for propagation in beef-extract broth. This procedure was followed in establishing the test orgainism.

1. Culture Stability

The culture maintained over a period of several months by the procedure described previously provided an orange-red pigmented organism of approximately 0.7 to 1.0 micron in diameter and 1.0 to 2.0 microns in length. The stability of the test bacterium was periodically affirmed under standard conditions of temperature and relative humidity (68° F and 65 per cent, respectively).

The bacterium S. marcescens ATCC 274 is known to be composed of multiple chromogenic and nonchromogenic variants. Under different cultural conditions, these variants exhibit differential stabilities, the pigmented variant showing the greatest stability.

The primary concern with the variants of S. marcescens ATCC 274 in relation to this investigation was the cultural characterization and maintenance of a standard bacterium as correlated with the standard atmospheric conditions mentioned above. This requirement became apparent when, after months of stable performance, the culture began to indicate changes, as evidenced by significant increases in k_t values--a reduced survival capacity under the standard atmospheric conditions. This changed response was at first thought to be a reflection of error in technique or in the mechanics of operation of the aerosol chamber. The problem was

approached with these factors in mind, including the possible physiological change in the test bacterium. Examination and testing eliminated all those factors under consideration except the bacterium, which was then studied in some detail.

2. Characterization of Stable Culture

The original isolation of the test bacterium was made on the basis of chromogenicity, but, with the manifested physiological alteration, a more definitive characterization of the bacterium in relation to the air-borne state became necessary. This primarily concerned the orange-red variant which was the original isolate. However, some work was done with the other color variants of the blood-red, pink, and white variety. Extensive bacteriological studies were beyond the intent of this project, and, since excellent work on this same strain of S. marcescens has been reported in the literature, efforts were restricted to securing information providing a basis for monitoring the test culture.

The current culture showed an aberrant response to the standard atmospheric conditions by a static k_t of 0.059 in contrast to the firm value of 0.034 established over a period of several months. Transfers were made into beef-extract broth from the previously inoculated nutrient agar stock slant which had been refrigerated without mineral oil overlay. The static k_t of the aerosol chamber was determined to be 0.075 showing a similar deterioration of the stock culture. An entirely new specimen of S. marcescens, ATCC 274, was obtained and transferred directly into beef-extract broth without preliminary screening of the chromogenic variants. The static k_t value obtained with this culture was 0.034. Although this culture exhibited the same response under standard atmospheric conditions as that previously established, it was questionable whether such a culture would

be inherently stable because of the chromogenic and nonchromogenic variants present. The variants were separated from the culture being used routinely, designated as Culture A, and from the newly received culture, designated as Culture E. The criteria of separation was the color of the colonies: BR (blood-red), R (orange-red), P (pink), and W (white). The culture medium was nutrient agar. The distribution of variants in the undifferentiated cultures and the subsequent behavior of these variants when carried through a number of transfers in beef-extract broth are shown in Table XII.

In order to determine the static $\underline{k}_t$ values, the variant isolates were tested in the aerosol chamber under the standard atmospheric condition. These results are tabulated in Table XIII. The standard static $\underline{k}_t$ is found within the range of response shown by E-R and E-P. The variant E-R was continued as the standard test organism, and, from 11 subsequent determinations, the static $\underline{k}_t$ value of 0.031 was obtained. The variant E-P was not used further because it was found that the more highly pigmented variants possess the greater cultural stability. Table XII indicates the incipient differentiation of variants of E-P at the sixth transfer in beef-extract broth.

Additional characterization of the stable test culture was sought in the study of possible morphological and dimensional variation by the use of phase and electron microscopy.

Examination of Culture A, which had evidenced an abnormal response to the standard atmospheric condition, showed a wide variation in cell size, ranging from 1 to 2 microns in length and 0.7 to 1.0 micron in width to 15 microns in length and 1.0 to 1.5 microns in width. Many of the longer filamentous-type cells showed loci of increased density to the electron beam of the electron microscope.

TABLE XII

DISTRIBUTION OF COLOR VARIANTS OF
S. marcescens, ATCC 274, AFTER FOUR DAYS AT 20° C

Number of Broth Transfers	Culture	Number of Various Colony Forms				Total
		BR	R	P	W	
100	A	157	797	43	3	1,000
20	E	0	1,431	27	2	1,500
1	A-BR	1,700	0	0	0	1,700
1	A-R	0	2,300	0	0	2,300
1	A-P	0	0	2,000	0	2,000
1	A-W	0	0	-----	1,500	1,500
1	E-BR	1,927	27	229	17	2,000
1	E-R	0	1,200	0	0	1,200
1	E-P	0	0	1,984	16	2,000
1	E-W	0	0	4	1,996	2,000
6	A-R	0	4,489	0	11	5,500
6	A-W	0	16	0	4,184	4,200
6	E-BR	303	3,338	115	44	3,800
6	E-R	0	10,970	1	29	11,000
6	E-P	3	254	2,692	51	3,000
6	E-W	0	0	51	4,949	5,000
45	A-R	0	1,010	15	150	1,175
45	A-W	0	0	11	800	811
45	E-R	0	1,200	0	0	1,200
45	E-P	0	955	162	76	1,193
45	E-W	0	0	112	1,000	1,112

TABLE XIII

DIE-AWAY RATES, AIR-BORNE VARIANTS OF S. marcescens, ATCC 274, AT 68° F, 65 PER CENT RELATIVE HUMIDITY

Number of Broth Transfers	Calory Forms							
	Blood-Red		Red		Pink		White	
	A†	E	A	E	A††	E	A	E
3	-	0.042	0.005	0.017	-	0.035	0.005	0.039
6	-	0.053	0.054	0.036	-	0.037	0.008	0.047
7	-	0.049	0.010	0.021	-	0.023	0.005	0.054
8-19	-	-----	-----	0.031	-	-----	-----	-----

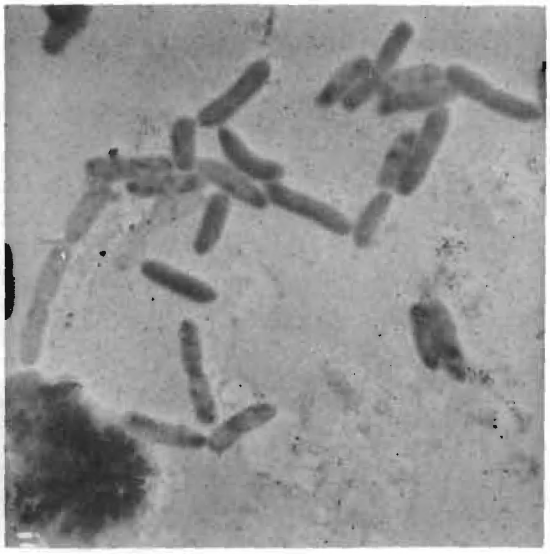
† Too few air-borne to obtain values.

†† After two isolations, cultures lost the pink color, became predominantly red and were discarded.

Culture E showed a more homogeneous population with cells having lengths ranging from 1.0 to 2.5 microns and widths ranging from 0.7 to 1.0 micron. Electron micrographs of this culture disclosed a uniform intracellular density.

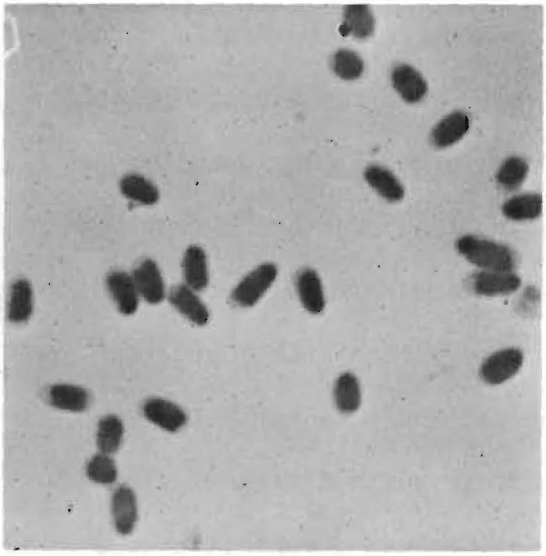
The separation of the variants from Culture A and Culture E provided a more detailed examination of the cell types as correlated with colony pigmentation. Electron photomicrographs of third-isolate variants from nutrient agar surface colonies show a significant morphological and size variation among the variants of Culture A. These variants also appear quite different from the corresponding variants of Culture E which does not, however, exhibit a correspondingly wide range of cellular differentiation among it variants. The pictures of the variants of Culture A are shown in Figure 16; those of Culture E, in Figure 17.

The correlated findings of morphology, cell dimension, and chromogenicity, together with the static $\underline{k}_t$ determinations, form the basis for monitoring the

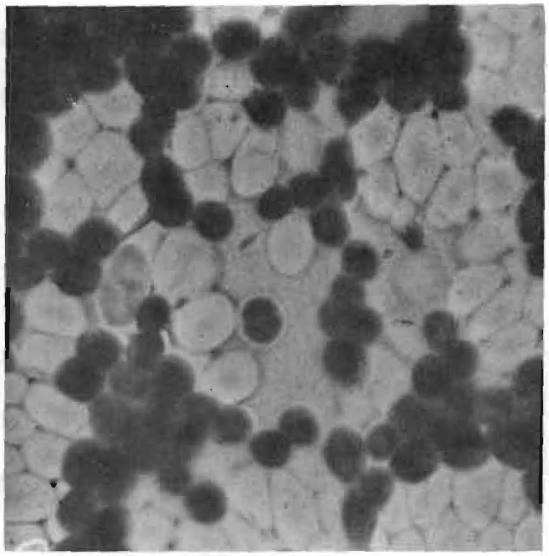


A-BR

A-R

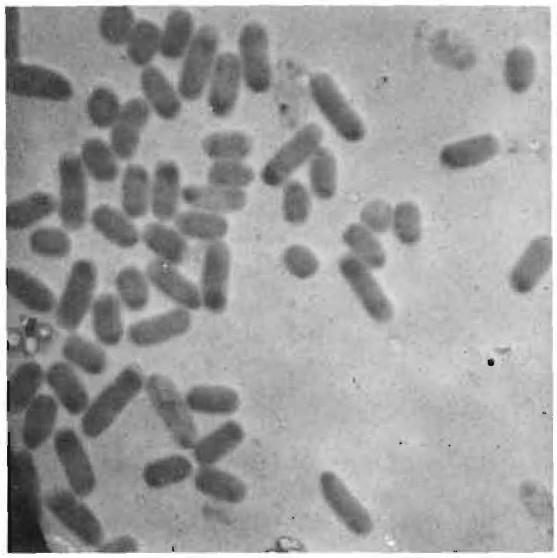


A-P

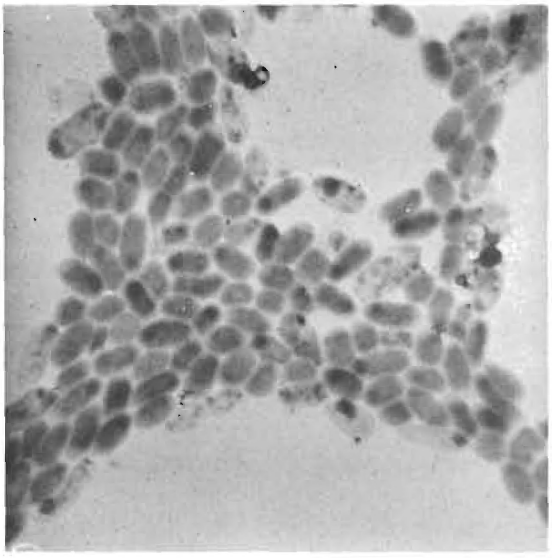


A-W

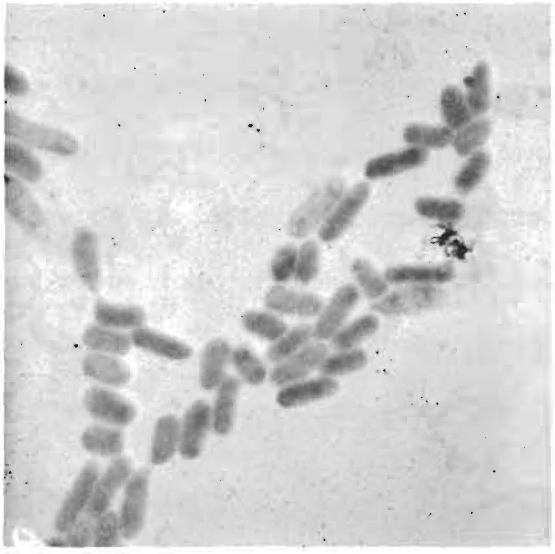
Figure 16. Electron Micrographs of the Variants of Culture A.



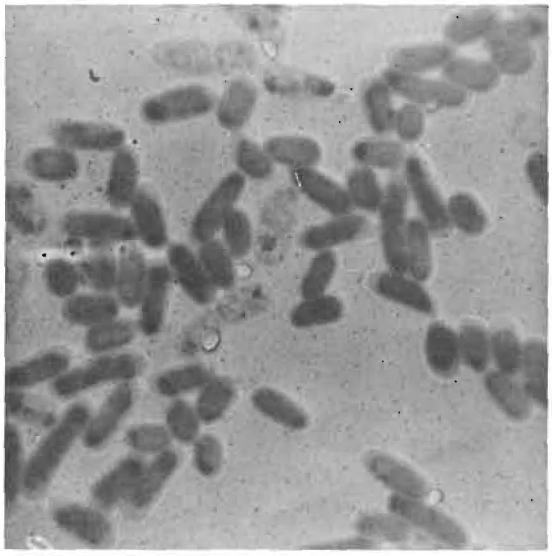
E-BR



E-R



E-P



E-W

Figure 17. Electron Micrographs of the Variants of Culture E.

test culture and enable a direct recovery of the test culture at such times when deterioration may be indicated. Storage of the variants on nutrient agar butts with mineral oil overlay at 5° C has proven satisfactory.

S. marcescens has been widely used as a test organism in the study of airborne microorganisms and has proven to be excellent for this purpose. However, as can be be deduced from the data presented, adequate monitoring of the particular variant being used is imperative if results are to be consistent. Although many other workers have used this organism, they have omitted the designation of the culture strain in reporting their results, and, therefore, it is impossible to determine if variations were encountered. Doubtless, other microorganisms used in similar studies have varying degrees of cultural stability and, therefore, exhibit differential responses when exposed to various atmospheres. The demonstrated differential responses among the isolated variants of S. marcescens, ATCC 274, serve to delineate the basic problems inherent in bacterial test organisms. They indicate that studies of the relative behavior of different genus and species should be extended to include the possible variants which may show as great a responsive difference as the different species exhibit. More detailed cultural studies may resolve some of the apparent disagreement in results reported by different workers who utilize, supposedly, the same organism.

V. STUDIES ON OPERATIONAL VARIABLES

A. Liquid-Impinger Samplers

In the preliminary phase of this work, the assaying of the viable constituents of a bacterial aerosol cloud was done by the impingement of the aerosol into a liquid medium by means of an orifice tube operated at critical velocity. The liquid medium consisted of a 0.2 per cent concentration of pharmaceutical gelatin (Pharmagel A - Pharmagel Corporation), in distilled, deionized water and was buffered to a pH of approximately 7 by the addition of 0.08 gram of dibasic sodium phosphate (anhydrous) per gram of gelatin. To prevent excessive frothing and subsequent loss of the medium during actual sampling, 0.5 ml of medicinal grade olive oil was added per 200 ml of sampler volume. Samplers of less than 200 ml received a corresponding lesser amount of olive oil per sampler.

1. Viability of S. marcescens in Sampler Medium

The duration of the sampling period for any one sampler depended upon the capacity of that particular sampler and the estimated bacterial aerosol concentration and hence might vary from a few minutes to almost an hour. The determination of the viability of the collected bacteria in this medium for varying periods of time was of prime import. To test the viability of S. marcescens in the liquid-impinger-type sampler, three different capacity samplers were used (28.3, 5, and 1 liter per minute). Each sampler contained, respectively, 400, 70, and 55 milliliters of 0.2 per cent of buffered gelatin solution, and the appropriate amount of olive oil to prevent frothing of the medium. A known number of bacteria was added to each sampler, and the samplers were operated at critical velocity in parallel vacuum connections. Aliquot volumes were removed from the samplers periodically during the operation, and viability determinations

by culture in nutrient agar were made. The results of the test are shown in Table XIV and represent the number of viable cells expressed as N x 10^7 per milliliter.

TABLE XIV

VIABILITY OF S. marcescens IN IMPINGER DURING A 60-MINUTE PERIOD†

Time (min)	Sampler Capacity 28.3 (l./min)	5 (l./min)	1 (l./min)
0	85	109	89
15	85	104	83
30	50	82	88
45	87	85	75
60	169	90	107
Average =	95	94	88
σ =	44	12	12
C. V. =	46	13	14

† Tabulated values = N x 10^7 per ml viable cells.

These results indicate a lack of any inimical effect of the collection medium on the collected cells. The variation observed in the results from 28.3-liters-per-minute sampler may be attributed to some extent to technique errors in subsequent dilution of the aliquot and plating; however, the principal source of variation is considered to be derived from the violent agitation and atomization produced by the high flow rate of sampler air. The results of experiments comparing critical-orifice samplers of different flow rates used in sampling a bacterial

aerosol cloud under dynamic conditions indicate a significant difference in the collection and/or loss of bacteria (Table XV). In these experiments, the liquid-sampler volume of 200 ml created an impingement bed 4 centimeters in depth, and the glass impinger tubes were positioned at different depths in the liquid. The 1-liter-per-minute impinger was positioned 2 centimeters below the surface, and the 12- to 28-liter-per-minute impingers were positioned 1 centimeter below the surface in order to present the impinging air jet with a liquid surface at all times during operation and to prevent impingement onto the glass surface of the containing bottle. The cause of the difference among the various capacity impingers cannot be determined from the data presented in Table XV. There appears to be a significant difference in the collection efficiency of the impingers, particularly between the 1-liter-per-minute and the 24- to 28-liter-per-minute sampler. Losses may occur as a result of the violent agitation produced by the high flow-rate impingers. A combination of factors is quite possible also. The demonstrated fact remains that, as the capacity of the impinger increases, the number of bacteria recoverable from the sampler decreases. This finding is quite pertinent in regard to the selection of a sampler for use in testing various liquid-impinger media.

These results show a general absence of any inimical effect of the gelatin collection medium on the subsequent cultural viability of the organism. The exposure period was within the time limits of sampling usually encountered for this work. The larger capacity samplers showed more variation in the bacterial counts, a fact perhaps related to the violent agitation of the impinger solution with attendant fluid loss.

TABLE XV

COMPARISON OF CRITICAL-ORIFICE SAMPLERS OF DIFFERENT FLOW RATES AT 70° F†

Per Cent Relative Humidity	Impinger Sampler Flow Rate (l./min)							
	1		12 to 15		20		24 to 28	
	Average Concentration of Bacteria Per Liter of Air							
	Count†	No. of Samples	Count†	No. of Samples	Count†	No. of Samples	Count†	No. of Samples
48	3470	(2)	2750	(1)	4630	(1)	2540	(2)
50	2180	(1)	1840	(1)	2050	(1)	1580	(1)
62	2110	(4)	2250	(6)	1865	(2)	1450	(4)
70	3160	(8)	2100	(2)	1875	(2)	1750	(2)

† Bacterial counts corrected to 100 x 10^7 organisms per milliliter.
Sampling time: 1 liter per minute: 20 minutes
12-28 liters per minute: 5 minutes

The 1-liter-per-minute sampler appears to be definitely superior to the 24- to 28-liter-per-minute sampler under the conditions of comparison. The cause of this difference is not apparent from the experimental evidence. It does appear, however, from a comparison of the data of Tables XIV and XV that the organisms once collected in the liquid medium are retained in the lower capacity sampler. Some loss caused by the high turbulence, and perhaps re-atomization, in the higher rate of flow impingers, viz, the 24- to 28-liter-per-minute samplers, may occur. The collection action of the critical-orifice impinger tube is dealt with more fully in the appendix.

The 1-liter-per-minute, critical-orifice, impinger tube in a 200-ml, liquid-impingement bed has proven to be a reliable bacterial aerosol-sampling device on the basis of examination and prolonged usage during the course of this work. The

principal variable, assuming constancy of other factors such as critical velocity of air jet and impinger volume, is the composition of the liquid-impingement medium, which is discussed in the following section.

2. Effect of Medium Composition on Collection of Air-borne S. marcescens

The comparative inter-sampler performance of 0.2 per cent buffered gelatin solution was very satisfactory. However, the question arose as to whether or not this medium, because of its chemical and/or physical composition, was actually demonstrating all of the potentially viable cells collected from a bacterial aerosol cloud. The production of a bacterial aerosol cloud from a single culture represents a finite number of individual organisms but an indeterminate distribution of organisms related to the adverse effects of varying atmospheric conditions. This bacterial aerosol cloud may be characterized, in one respect, as a population possessing what might be termed a differential viability potential. Such a population would contain cells showing only a negligible dispersion effect into air and a gradation of cells showing varying degrees of injury, a reflection of their viability potential. If the assumption is made that this injury involves a reversible mechanism and that the demonstration of the injured cell is dependent upon the reversal of this mechanism, a reconsideration of the biologically significant components of the collection medium becomes necessary.

Bacteria disinfection studies have demonstrated a differential response of organisms to various disinfecting agents, such a response being exhibited by using various nutrient media employed after injury to the cell. An analogous situation was considered to exist in the bacterial aerosol cloud. To examine this problem, a series of impinger media of different compositions was used in assaying the bacterial aerosol cloud, and the results of these experiments are shown in Table

XVI. A detailed investigation of the reversal effects was beyond the design of this experiment since the primary purpose was to determine whether the 0.2 per cent gelatin solution was exhibiting all cells potentially surviving within the sampling period. The results affirmed that many of the injured cells within the group of lower viability potential have been lost because of the absence, in the collecting medium, of suitable nutrient constituents of such nature as to reverse or terminate the injury mechanism.

The selection of brain-heart infusion as one of the test constituents of the collection medium was based on the fact that perhaps the injured cells of low viability potential possess nutritive requirements analogous to those of the fastidious pathogenic microorganisms, which have been shown to be quite cultivatable in this medium. In addition to the significantly increased collection efficiency, this medium has the further practical advantage of being constituted of a minimum number of standard commercially available items and requires no special technique of preparation.

Concomitant with the studies on the effect of various impinger media on the collection of air-borne S. marcescens, comparative studies were made on DOW AF (a water dilutable dispersion of Dow Corning Antifoam A silicone defoamer) and olive oil added to the impingers as antifoaming agents. Table XVII shows that the differences exhibited by impinger media have no effect on the numbers of cells collected. The DOW AF antifoaming agent visually appears to be more efficient in dampening foam formation, and it has the additional advantages of being more easily dispensed into the individual samplers and of not producing objectionable effects in the solid plating medium, as do the oil globules.

TABLE XVI

EFFECT OF VARIOUS IMPINGER MEDIA ON
COLLECTION OF AIR-BORNE S. marcescens

No.	Medium	Average Concentration of Bacteria Per Liter of Air		Concentration Adjusted to 100 x 10^7/ml Culture Count	
		Run 1†	Run 2††	Run 1	Run 2
1	Gel. (DOW AF)	3675	2410	4950	4150
2	Gel. (Oil)	3650	2145	4930	3690
3	Gel.-Broth (DOW AF)	6000	3165	8100	5460
4	Gel.-Broth (Oil)	5125	3400	6900	5860
5	Broth (DOW AF)	6000	3230	8100	5570
6	Broth (Oil)	3950	1966	5340	3390
7	Gel.-B.H. (DOW AF)	8550	5005	11550	8700
8	Gel.-Lact. (Oil)	6200	----	8370	----
9	Gel.-Prot. Pep.-Yeast Extr. (Oil)	5050	----	6830	----
10	Gel.-NaCl-Lactose (Oil)	----	3480	----	6000
11	Gel.-Prot. Pep.-NaCl-Yeast Extr. (Oil)	----	5280	----	9130

† Run 1 - DB 67° F, DP 59° F, RH 74 per cent -- (Average of 2 determinations).

†† Run 2 - DB 70° F, DP 53° F, RH 55 per cent -- (Average of 3 determinations).

Composition of Media

1 and 2 - 0.2 per cent gelatin solution (Pharmagel A), 0.08 gram Na_2HPO_4, anhydrous, per gram of gelatin; pH 6.95.
3 and 4 - 0.2 per cent Pharmagel A (buffered) in 0.3 per cent beef-extract broth; pH 6.98.
5 and 6 - 0.3 per cent beef-extract broth; pH 6.8
7 - 0.2 per cent Pharmagel A (buffered) in 3.7 per cent brain-heart infusion; pH 7.2.
8 - 0.2 per cent Pharmagel A (buffered), 0.25 per cent NaCl, 0.5 per cent lactose; pH 6.65.
9 - 0.2 per cent Pharmagel A (buffered), 2 per cent proteose peptone, 0.3 per cent yeast extract, 0.25 per cent NaCl; pH adjusted with potassium hydroxide (10 per cent) to pH 7.1.
10 - 0.2 per cent Pharmagel A (buffered), 0.25 per cent NaCl, 0.5 per cent lactose.
11 - 0.2 per cent Pharmagel A (buffered), 2 per cent proteose peptone, 0.3 per cent yeast extract, 0.25 per cent NaCl; pH adjusted with potassium hydroxide (10 per cent) to pH 6.9.

DOW AF - loop inoculum per impinger sample.
Oil - Olive oil - 0.5 ml per impinger sample.

TABLE XVII

EXAMINATION OF DIFFERENCES
EXHIBITED BY IMPINGER MEDIA

Atmospheric Conditions: DB 70° F, DP 53° F, RH 55 per cent
Culture: S. marcescens ATCC 274

Medium	Averages ($\bar{x}$)	σ	σ_D	$\bar{x}_1 - \bar{x}_2$	$3\sigma_D$	Significance
DOW AF versus Olive Oil (0.2 per cent gelatin)						
DOW AF	2410	134	112	320	336	-
Olive Oil	2090	104				
DOW AF versus Olive Oil (0.3 per cent Beef-Extract Broth)						
DOW AF	3306	355	235	466	705	-
Olive Oil	2840	202				
Gel. versus Gel.-B.H.						
Gelatin	2218	234	141	3038	423	+
Gel.-B.H.	5256	164				
Gel. versus Broth						
Gelatin	2218	234	171	855	513	+
Broth	3073	332				
Broth versus Gel.-Broth						
Broth	3073	332	211	469	633	-
Gel.-Broth	3542	362				

Note - All σ determinations subsequent to DOW versus oil included averages of DOW and oil.

The large volumes of impinger solution required led to a study of possible material economy in regard to the brain-heart infusion. To determine the effect of various concentrations of brain-heart infusion in 0.2 per cent gelatin solution (buffered), an experiment was made using impinger samplers of 0.8, 1.6, and

3.2 per cent concentration. The results of this experiment indicated that 1.6 per cent brain-heart infusion was the minimum concentration of choice. Although the 0.8 per cent concentration yielded more viable cells than plain 0.2 per cent gelatin, the numbers were slightly less than those from the chosen concentration. Culture of the stock culture used in this experiment in nutrient agar and nutrient agar enriched with brain-heart infusion (1.6 per cent) did not produce disparate cell counts.

A further study of the relative values of brain-heart-infusion gelatin solution and plain-gelatin solution showed that the k_t values were unchanged. This value is determined from the $\log_{10}$ of the numbers of bacteria entering the chamber less the $\log_{10}$ of the numbers of bacteria leaving the chamber divided by the retention time of 5 minutes. This result (Table XVIII) may be considered to indicate that the spectrum of differential viability potential remains constant, the brain-heart-infusion gelatin medium merely increasing the range of collection of cells within the spectrum, and that the enhanced collection effect of this medium is manifested quantitatively and not qualitatively.

3. Examination of Sampler Variation

In addition to the demonstrated quantitative differential of collection between the brain-heart gelatin and the plain-gelatin impinger solutions, pertinent information is desirable as to the extent of variation among samplers operated concomitantly and, also, the variation existing among data derived from experiments performed on different days, e.g., the degree of reproducibility of experimental conditions. Data were compiled at 70° and 80° F (DB) and at various relative humidities. The sampling media were brain-heart gelatin and plain-gelatin solutions. Each sampler contained a 200-ml liquid volume with a

TABLE XVIII

COMPARISON OF PLAIN-GELATIN AND BRAIN-HEART-GELATIN, LIQUID-IMPINGER SOLUTIONS

Culture: S. marcescens, ATCC 274, E
Atmospheric Conditions: DB 70° F, RH 60 per cent

	Plain Gelatin			Brain-Heart Gelatin		
	Inlet	Exit	k_5	Inlet	Exit	k_5
Average =	5790	2650	0.068	9700	5038	0.057
σ =	1805	684		1160	1110	
C. V. =	31	26		12	22	
N =	4	4		4	4	

Inlet: Gel. $(\bar{x})$ - B.H. Gel. $(\bar{x})$ = 3910 $\quad \sigma_{D\ mean}$ = 1070

Outlet: Gel. $(\bar{x})$ - B.H. Gel. $(\bar{x})$ = 2388 $\quad \sigma_{D\ mean}$ = 652

critical-orifice impinger tube and operated for 10 minutes (brain-heart gelatin) and 20 minutes (plain gelatin). The bacterial content of the collected sample was plated in triplicate for cell enumeration. Table XIX shows the compilation of these results at 70° F, while Table XX shows the results at 80° F.

The results at 70° F indicate that the variation among concomittantly exposed samples, either brain-heart gelatin or plain gelatin, is within acceptable limits. The establishment of 15 per cent limits (coefficient of variation = 15 per cent) to include errors inherent in aliquoting and plating of the solution, should be reasonable. However, if the composite data derived from experiments performed on different days are considered, it will be seen that the plain-gelatin impinger samplers show an excessively high variation regardless of the relative humidity under which the samples were taken. These results may mean that (1) the character of the bacterial aerosol is different from one experimental date to another,

TABLE XIX

COMPARISON OF VARIANCES BETWEEN BRAIN-HEART-GELATIN AND PLAIN GELATIN AT 70° F

Culture: S. marcescens, E-R

Per Cent Relative Humidity	Date and Run No.†	Brain Heart Gelatin				Plain Gelatin			
		Air†† (conc/l.)	N	σ	C.V.	Air†† (conc/l.)	N	σ	C.V.
20	11/3-(1)-C	13315	6	960	7.2	10041	6	1038	10
	11/9-(2)-C	9970	3	633	6.0	5343	3	275	5.2
	(2)-E	9340	3	339	3.6	-----	-	----	----
	11/4-(3)-C	10050	3	378	3.0	4200	3	272	6
	(3)-E	8973	3	466	5.0	-----	-	----	----
60	10/6-(1)-C	4276	3	368	8.6	2053	3	35	1.7
	10/22-(2)-C	9240	3	344	3.7	4423	3	110	2.5
	10/29-(3)-C	5766	3	104	1.8	3430	3	125	3.6
83	11/6-(1)-C	20190	5	2690	13.0	6521	6	933	14
	(1)-E	19991	6	3143	15.7	-----	-	----	----
	11/17-(2)-C	17216	3	1402	8.1	2660	3	130	4.9
	(2)-E	18866	3	1501	9.9	-----	-	----	----

† Run No. indicates concomitant samples. The letters C and E denote that the aerosol sample was taken from the Chamber or Exit of the chamber.

†† Bacterial concentration per liter corrected to 100 x 10^7 per ml of original culture.

e.g., the technique has a low factor of reproducibility, or (2) that the variation is due to undetermined characteristics of the collection medium. The possibility also exists that the variation is due to a combination of these factors. The low variation in the brain-heart-gelatin samples indicates that the first hypothesis should be rejected and that the high variation seen among the plain-gelatin samples is due to factors in the collection medium itself. The wide variation among the total numbers collected by the brain-heart-gelatin samples at 60 per cent

TABLE XX

COMPARISON OF VARIANCES BETWEEN BRAIN-HEART-GELATIN AND PLAIN GELATIN AT 80° F

Culture: S. marcescens, E-R

Per Cent Relative Humidity	Date and Run No.†	Brain-Heart-Gelatin Air†† (conc/l.)	N	σ	C.V.	Plain Gelatin Air†† (conc/l.)	N	σ	C.V.
20	11/23-(1)-C	8828	6	1260	14.3	----	-	----	----
	(1)-E	7953	3	383	4.8	3195	2	355	11
	11/23-(2)-C	8800	3	304	3.4	----	-	----	----
	(2)-E	7293	3	912	12.5	----	-	----	----
44	11/10-(1)-C	5120	3	416	8	2236	3	168	7.5
	(1)-E	4953	3	100	2	----	-	----	----
	11/10-(2)-C	6066	3	375	6.2	2636	3	238	9
	(2)-E	5893	3	306	5.2	----	-	----	----
78	11/11-(1)-C	15100	3	1531	10	8873	3	172	1.9
	(1)-E	13283	3	202	1.5	----	-	----	----
	11/11-(2)-C	15416	3	2008	13.5	8900	3	1465	16
	(2)-E	15383	3	1211	7.8	----	-	----	----

† Run No. indicates concomittant samples. The letters C and E denote that the aerosol sample was taken from the Chamber or Exit of the chamber.

†† Bacterial concentration per liter corrected to 100 x 10^7 per ml of original culture.

relative humidity indicates a possible disagreement with this conclusion. An assumed basis has been derived and is developed in later paragraphs which seek to explain this apparent departure from the collection characteristics of the brain-heart-gelatin solution. It should be noted that, at 60 per cent relative humidity, the lowest aerial concentration of bacteria exists. The net k for 20, 60, and 82 per cent relative humidity is 0.004, 0.026, and 0.013, respectively [Progress Report G-2771 (C-2, 4/1/53 - 2/1/54]. These findings indicate an area of investigation which might elucidate the observed variation at 60 per cent relative humidity, but, unfortunately, the data available at present are insufficient for a definite conclusion.

A further examination of the total numbers of bacteria collected under any relative humidity indicates that the difference in numbers collected by the brain-heart-gelatin solution and the plain gelatin is a significant difference and not a difference due only to chance. The differential of numbers collected at 83 per cent relative humidity becomes quite large.

A similar comparison of the variances between brain-heart-gelatin and plain-gelatin impinger solutions at 80° F, (Table XX) does not show any significant variation among samples taken concomitantly. There were no data available to determine the reproducibility of conditions by sampling of the aerosol cloud under the same condition of atmosphere at 80° F, but on different dates, as indicated in Table XIX, the number of samples taken with plain-gelatin impingers is insufficient for comparison, although it is of value for general comparative trends. At 80° F, the brain-heart-gelatin solution continues to a significantly greater number of collected organisms than does the plain-gelatin solution.

Relative to the data presented in Tables XIX and XX, there are several pertinent points which cannot be omitted from this study of the variances between brain-heart-gelatin and plain-gelatin impinger solutions. These data referred to were derived from experiments done to establish the standard cultural variant of S. marcescens, ATCC 274, isolated from a culture slant received during the first part of September 1954. The procedure of isolation (as outlined under Chapter IV-B 2) was completed on September 30, 1954, and the beef-extract-broth cultures of the standard test variant, E-R were started.

The early results, obtained during the month of October (Table XIX-70° F-RH 60 per cent), indicate the instability of the culture as evidenced by the

disparate numbers of organisms collected per liter of air and the variation resulting therefrom. This presumed cultural instability may be a large factor in producing the high coefficient of variation among the plain-gelatin impingers at 70° F, as the medium has been shown to give a lower collection efficiency than brain-heart-gelatin impinger solution. This lowered efficiency is based on a hypothesis of a differential viability potential, previously postulated, reflected most frequently in the plain-gelatin impinger solution. As in the above case, the differential would be increased or shown to be more variable in the case of an incompletely stabilized test culture. Under the most adverse atmospheric conditions, this same variation could, conceivably, appear in the brain-heart-gelatin impingers as shown in Table XIX at 60 per cent relative humidity.

From the data presented, it can be tentatively concluded that the brain-heart-gelatin impinger solution results not only in larger numbers of organisms collected but also in increased repetitive performance having less susceptibility to test culture variation than the plain-gelatin solution.

4. Temperature of Liquid Impinger

Since the studies on air-borne bacteria were conducted under controlled conditions, all equipment used in the experiments is at the same temperature during any given period of experimentation. In a general consideration of the factors influencing the sampling of bacterial aerosol clouds, the question arises as to what influence the temperature of the liquid sampler may have on the collection of bacteria in determining the effects of various atmospheric temperatures and relative humidities on air-borne bacteria. The premise of this thought is that if the temperature of the liquid sampler has an influence on the bacteria

collected, this influence would represent an extraneous factor not directly related to the effect of various atmospheric conditions on air-borne bacteria.

An investigation of this postulated factor consisted of maintaining brain-heart-gelatin, liquid-impinger (critical-orifice) samplers at different temperatures during the sampling of an aerosol of S. marcescens, ATCC 274, E-R, in the main aerosol chamber. The prevailing conditions were 70° F and 60 per cent relative humidity. These tests were performed on two successive days. The liquid impingers were operated for periods of 5 and 10 minutes, the length of sampling time not appearing to be a factor. Three concomitant samples were taken from the chamber inlet as well as from the chamber exit. All samples were plated in triplicate directly from the sampler into the routine medium of tryptone-glucose-extract agar, buffered, and with NaCl added.

The results of this study are presented in Table XXI and indicate that there is no significant effect of sampler temperature in the range of 37° to 82° F on the numbers of bacteria collected. Samples maintained in the temperature range of 90° to 130° F show a decided depression of number of bacteria collected and a significant coefficient of variation among the samples taken, a degree of variation not found in the 37° to 82° F temperature group. The average $\underline{k}_t$, derived from the $\log_{10}$ inlet - $\log_{10}$ exit divided by 5 minutes (equilibration time), of all samples treated as a unit population gradually increases in magnitude with sampler temperature.

In view of the large coefficient of variation shown by samplers in the temperature range of 90° to 130° F, it is of interest to determine if this order of variation exists relative to the $\underline{k}_t$ values determined from concomitantly exposed groups of samplers, viz, whether the $\underline{k}_t$ factor is correlated with the

TABLE XXI

EFFECT OF LIQUID-IMPINGER-SAMPLER TEMPERATURE ON COLLECTION OF AIR-BORNE S. marcescens, ATCC 274, E-R, AT 70° F, 60 PER CENT RELATIVE HUMIDITY

	37° to 48°F		56° F		74° to 82° F		90° to 130° F	
	Inlet	Exit	Inlet	Exit	Inlet	Exit	Inlet	Exit
Conc/l. =	8972	5710	8910	5166	10483	5541	6015	2945
N =	12	11	3	3	6	6	12	12
σ =	1983	810	1506	471	1599	678	2515	1308
C.V. =	22	14.2	16.8	9.1	15.3	12.2	41.8	44
k_t † =	0.034		0.047		0.055		0.062	
k_t †† =	0.039		0.047		0.055		0.064	
N =	4		1		2		4	
σ =	0.006		-----		0.002		0.013	
C.V. =	15.4		-----		3.6		20	

† Derived from composite average.

†† Derived from groups of three concomitantly exposed samples.

total numbers of viable cells collected. The k_t was determined for each group of three inlet and three exit samples as previously described and an average and a deviation was derived. These data indicate that the essentially same k_t as determined from composite averages of inlet and exit samples does not show a large coefficient of variation relative to the temperature of the collecting impinger liquid. Because of the number of samples involved, this comparison is limited principally to the 37° to 48° F, and 90° to 130° temperature groups. The large

variation in numbers of viable cells collected in the impingers at 90° to 130° F, apparently is independent of the factors composing the $\underline{k}_t$ value to a significant degree.

These data indicate some parameters in the problem of sampling the microorganism content of the air. Under conditions of laboratory experimentation, the conditions of aerosol production using pure culture result in a very wide range of susceptibility of individual cells within the cloud population. As has been shown, the relative effects ($\underline{k}$) of the atmosphere on this population remain unchanged, but the effects on absolute numbers appear definitely established in the range of the atmospheric conditions studies. This quantitative effect may be correlated with atmospheric temperature, and the lower temperatures may show less disparate quantitation of numbers of bacteria collected in various media as the inimical effects of the atmosphere are reduced. This might be suggested from the results of maintaining impinger samplers at various temperatures.

The rate of sampling using an impinger of constant volume, 200 ml in the majority of the experiments reported, becomes a significant factor as the rate of sampling is increased beyond a certain range. While the viability of the bacteria contained in the sampler liquid appears unaffected during a reasonable time of sampling, the higher sampling rates indicate a failure of particle collection or a possible loss of organisms after collection. This loss, if present, is probably mechanical and is derived form the violent agitation and atomization of the impingement liquid. For the higher rate samplers this could doubtlessly be overcome by increasing the total liquid volume and by altering the geometry of the impingement bed. The possibility of reduced intrinsic efficiency of the high floor rate samplers must be borne in mind also. A resolution of this reduced number of organisms collected must come from more detailed experimentation.

There are, then, several factors relating to the collection of air-borne microorganisms, and these factors doubtlessly bear an interrelationship which includes the chemical components of the collection medium, rate of collection, and temperature. The temperature factor may relate to atmospheric and/or sampler temperature. A further and more detailed study of these points would provide a contribution to the problem of air-sampling and possibly a better insight into the nature of the response of bacteria to atmospheric conditions.

5. Standard Liquid-Impinger Medium

As the result of the studies on liquid-impinger media, the standard impinger composition and volume is as follows: brain-heart infusion, 16 grams; gelatin (Pharmagel A), 2 grams; dibasic sodium phosphate, anhydrous, 0.16 gram; distilled, deionized water, 1 liter. The gelatin solution (buffered) is first heated sufficiently to effect solution after which the brain-heart infusion is added. The volume of impinger medium in each sampler is 200 ml placed in a standard quart milk bottle. One culture-loop inoculum of DOW AF antifoaming agent is added and the sample is sterilized for 15 minutes under 15 psi of steam. The surface area (7.5 cm) and the depth (4 cm) of the liquid-impingement bed formed by 200 ml in the quart milk bottle were of dimensions which accommodated impinger velocities up to 10 liters per minute. The significance of the impingement velocity-liquid impingement bed relationship is considered elsewhere. Unless otherwise noted, this accepted standard impinger sampler was used throughout this work.

B. The Relation Between Dynamic and Static Values of the Die-Away

In the period immediately following the completion of the second progress report for this project (Progress Report, G-2771-C2), a series of studies was undertaken to determine more exactly the effect of temperature on the survival

of air-borne S. marcescens. During the course of these investigations, data were collected which appeared to indicate a marked difference between the values of die-away calculated from observations made using liquid aerial samplers in dynamic runs and those from observations made using settling plates in static runs. However, these studies were made during the period when the stock culture had changed its identity, and the data were found to be of doubtful value. A limited series of runs was subsequently made on the re-established culture in order to determine whether or not there is any significant difference between die-away values determined by the two methods. The results of this study are shown in Table XXII.

In the range from 68° to 71° F, there seems to be little if any significant difference in the k values determined either dynamically or statically. However, the determinations made at 80° F seem to indicate that, at 42 and 76 per cent relative humidity, the k values determined statically are greater than those determined dynamically. The same seems true for the single observation at 90° F. This finding is in general agreement with that noted earlier, on the basis of data of doubtful value.

In considering the possible causes of this difference between die-away values determined by the two different methods, the values determined dynamically are more likely to be correct. This conclusion is based on the general assumption underlying the interpretation of bacteriological death studies--that barring gross contamination and reproduction of the organism, the technique demonstrating the greatest number of viable organisms is most likely to be the best one. In other words, there numerous reasons why a technique can cause the apparent loss of viable organisms, and it is difficult (again barring gross

contamination and reproduction of the organism) to explain how an increased number of bacteria can be found. Under the conditions of operation, both gross contamination and increase by reproduction can be discounted. Therefore, it must be concluded that under the conditions shown in Table XXII, where a higher value for k is indicated as the result of static studies, the fault lies with these studies.

TABLE XXII

THE RELATION BETWEEN VALUES OF DIE-AWAY DETERMINED DYNAMICALLY AND STATICALLY IN THE MAIN AEROSOL CHAMBER (E-R CULTURE)

Atmospheric Conditions		Average Die-Away Values†	
Temperature (°F)	Relative Humidity (%)	Dynamic†† Conditions	Static Conditions
68	19	0.025	0.025
67	20	0.026	0.027
70	60	0.031	0.026
69	61	0.040	0.036
71	84	0.009	0.008
80	19	0.016	0.023
80	33	0.068	0.068
80	42	0.054	0.094
80	76	0.004	0.045
90	14	0.011	0.043

† The result of at least three observations.

†† Determined from data obtained through the use of sampling fluid enriched with brain-heart infusion.

Possibly, some failure of the settling plates to provide adequate conditions for the continued survival and growth of those organisms which settled upon them accounts for the differences described above. Because of limitations of time, this point has not been pursued further. However, it does warrant further study, because it indicates a very definite limitation on the use of settling plates and any type of aerial sampler employing solid nutrient surfaces for aerobiological studies.

C. The Response of Air-borne Organisms to a Sudden Change in Relative Humidity

The studies on the effect of chemicals on air-borne bacteria were carried out at 68° F under various relative humidities. During the course of this work with the aerosol cylinders, several hundred samples were collected at the inlet to the cylinders. Over 150 such samples were taken after the installation of the mixing system which diluted the prechamber output with air of a different dew point prior to entering the inlet to the cylinder system. Because the prechamber was maintained at 68° F at all times and was always operated in precisely the same manner, the output of the prechamber from day-to-day was essentially constant, varying only with the variations in culture count. Therefore, these inlet samples should have yielded comparable values for all the runs if reduced to a common culture count. However, preliminary analysis of the data from some of the runs indicated a possible difference associated with differences in relative humidity.

Data analyses were made on all the samples taken from the inlet to the aerosol cylinders under conditions where the prechamber output was adjusted to the desired relative humidity prior to reaching the inlet to the cylinders. These data were considered as representing the condition found when a bacterial aerosol

is suddenly transported from near saturation humidity to a lower relative humidity. The change from the near saturation humidity existing in the prechamber to the lower relative humidity takes places quickly when the 6.4 liters of prechamber output is mixed with 22 liters of air having a lower dew point. The time of passage of this mixture through the mixing cones and, thence, to the inlet to the cylinder system is less than 1 minute.

The samples taken at the inlet to the cylinder system under the conditions outlined were adjusted to a common culture count of 100 x 10^7/ml and expressed as thousands of viable organisms per liter of air. Then, they were grouped according to the final relative humidity achieved for the various runs; the results were averaged, and the standard error of the average of each group was determined. The resultant information is shown in Table XXIII. Included in this table are values for the average $\underline{k}$ for each relative humidity. Also included are the corrected average numbers which would exist if the passage through the mixers required 1 minute, during which time the appropriate death rate effected losses in the same manner as in the cylinder or chamber. No correction was made for losses by settling as the entire flow is upward at a rate sufficient to maintain all particles air-borne. Even the greatest difference observed in particle size was calculated to yield a maximum difference of less than 5 per cent, if losses occurred by settling. Therefore, the possible influence of settling losses was considered negligible and disregarded. The values given in the last column of this table are the minimum per cent losses due to unknown causes, the greatest number recovered (25 per cent relative humidity) having been set at 100 per cent.

The information given in Table XXIII appears to indicate that the apparent losses occurring in the mixers (when the bacterial aerosol is suddenly changed

TABLE XXIII

AVERAGE NUMBERS OF VIABLE AIR-BORNE ORGANISMS† COLLECTED FROM THE ENTRANCE TO THE AEROSOL CYLINDERS AT VARIOUS RELATIVE HUMIDITIES AT 68 ° F

Per Cent Relative Humidity	Average Numbers†† of Viable Organisms Per Liter x 10^{-3}	Standard Error of Average	Number of Samples	Average k	Average Number Corrected for Death Losses	Per Cent Loss Due to Other Causes
25	810	24.5	43	0.02	848	0
30	635	24.0	11	0.025	673	22
50	695	21.0	97	0.025	736	13
60	625	15.0	9	0.03	685	19
65	495	30.0	12	0.035	536	37
75	540	26.0	17	0.02	566	33
80	588	19.5	69	0.01	602	29
90	525	16.0	16	0.01	537	37

† S. marcescens, E-R/O - primary form of ATCC 274.

†† Corrected to a 100 x 10^7 per ml culture.

from near saturation to lower relative humidities) are related to the relative humidity achieved in the mixers. The greatest losses occur when the final relative humidity is high; the lowest losses occur when the final relative humidity is low. In examining the values presented in this table for significant differences, it was found that there is a significant difference between the average number demonstrated at 25 per cent relative humidity and the averages for all other relative humidities. Further, there is no significant difference between the averages for 30, 50, and 60 per cent relative humidity. There is a significant difference between the values for 60 and 65 per cent relative humidity, indicating a greater loss at 65 per cent relative humidity. Among the values for 75, 80, and 90 per cent relative humidity, there is no significant difference. The value for 80 per cent relative humidity is significantly larger than is the value for 65 per cent, indicating that, at humidities above the 65 to 75 per cent level, there is less loss than at 65 per cent relative humidities. However, the fact that there is a significant difference between the average number recovered at 25 per cent relative humidity and those recovered at all other humdidities shows that the greatest recovery is at the lowest humidity.

The data in Table XXIII are interpreted as showing that, under the conditions of these experiments, there is a steady increase in the unexplained losses as the relative humidity increases (with the exception of the larger losses at 65 per cent). Having ruled out losses by settling and by death as the possible explanation, consideration was given to variations in sampling effectiveness. Such external information as was available was interpreted as being contradictory to this idea. For example, if changing relative humidity had played a similar role in the experiments where air samples were taken from the chamber or

cylinders using samplers containing fluid at temperatures lower or higher than that of the chamber or cylinder, then the samples collected in fluid at a temperature lower than that of the chamber or cylinder would have exhibited lower recoveries than would those having fluid at a temperature higher than that of the chamber or cylinder. This would be the case because the relative humidity of a cooled air sample will increase, just as the relative humidity of a warmed air sample will decrease. The fact that increasing the temperature of the sampling fluid above 80° F caused a sharp decrease in the numbers recovered seems to illustrate that sampling effectiveness is not involved in the phenomenon of losses in mixing the output of the prechamber at various relative humidities.

Consideration of the condition of the air-borne particle casts further doubt on the idea that variation in sampling effectiveness is the cause of the differences in numbers recovered at various relative humidities under these conditions. At the higher humidities, the particle contains more water than it does at lower humidities, and is, therefore, definitely larger. Both of these factors would indicate that the greatest effectiveness of sampling occurs under conditions of the highest humidity. However, for the case at hand, the highest numbers are demonstrated at the lowest humidity.

The factors of sampling effectiveness, the losses caused by settling, or the effect of the ordinary rate of death for the particular humidity having been examined carefully and eliminated, it can be concluded that the differences in average numbers shown in Table XXIII are entirely due to the sudden change from a humidity of near saturation to some lower relative humidity. In other words, these appear to be losses occurring during the process of equilibration. The statement is often made in the literature that the rate of drying affects the

survival of air-borne organisms and that the more rapid the rate, the better the survival, except at some intermediate or critical value of relative humidity. This statement is usually offered as an explanation of the effect of relative humidity on the survival of air-borne bacteria. However, because the death rates for organisms given in this report are always determined in the chamber or cylinders after a time period more than sufficient for equilibration of the organism to the particular conditions, this explanation has been found inadequate. That is to say, the data reported in Progress Report B-100, G-2771, C-2 (as well as those for k in Table XXIII) showed that intermediate relative humidities were more lethal to air-borne organisms, after they had equilibrated to the particular humidity, than were the higher or lower humidities. Therefore, rates of drying could not be involved in this survival after equilibration effect. However, in the case of sudden change in relative humidity, this explanation does appear to be applicable.

It thus appears reasonable that the data in Table XXIII for the average numbers demonstrated immediately after the prechamber output is mixed with air having a lower dew point can be interpreted as showing that the rate of drying of a bacterial aerosol does affect the survival of such bacteria. However, this phenomenon appears to occur only when bacterial aerosols are suddenly moved into a different humidity condition and does not continue to occur following equilibration. In other words, when the relative humidity of the air containing the bacterial aerosol is suddenly lowered from near saturation, the greater the lowering, the more rapid the rate of drying of the bacterial particle and the greater the probability that the bacteria will survive this equilibration process. The concept of a "critical humidity" seems applicable to the exceptionally great

losses due to equilibration to 65 per cent relative humidity. That is, increasing rate of drying is favorable to survival, but increasing solid concentration around the bacterium is unfavorable to survival; these two factors are at maximum unbalance at 65 per cent relative humidity.

Not only do the current findings lend support to a previously postulated concept in dealing with equilibration losses, but they may be helpful in explaining some of the published differences as to the effect of relative humidity on the survival of air-borne bacteria. In terms of survival during equilibration, the lower humidities have the least hostile effect; in terms of survival after equilibration, the higher humidities have the least hostile effect. In both instances, the intermediate relative humidities have the greatest hostile effect on the survival of the air-borne organism. Thus, depending upon the method of study employed, it would be possible to obtain results which showed that either high relative humidity or low relative humidity is the more favorable for the survival of air-borne organisms. In either case, the intermediate humidities would be described as most lethal.

D. The Effect of Relative Humidity on the Survival of Air-borne Organisms

In the previous report (G-2771, C-2), a large body of data were presented which showed that intermediate humidities were definitely more lethal to air-borne S. marcescens than are the very high or very low relative humidities. This was shown to be true for the 65° to 90° F range, with increasing temperature showing an increased lethal effect. Because it was recognized that the test organism shows considerable cultural instability, the die-away values for the culture were often redetermined. The total averages for these redetermined values are shown in Table XXIV. Data shown in this table were taken from both dynamic and static

runs. The averages shown in Table XXIV are in agreement with the earlier information, except in respect to the findings at 20 per cent relative humidity. The present results show a larger rate of die-away at this relative humidity than was found earlier. This change may be due to possible differences between the cultures. In the earlier work, culture differentiation was not carried out in as exacting a manner as in the more recent studies.

TABLE XXIV

THE RELATION BETWEEN DEATH RATE OF AIR-BORNE S. *marcescens* (E-R) AND RELATIVE HUMIDITY AT 68° F

Per Cent Relative Humidity	Average† Net k Due to Death	Standard Error of the Average	Coefficient of Variation	Number of Runs
20	0.0302	0.0005	4.6	8
57	0.0267	0.0003	2.7	6
61	0.0316	0.0019	11.9	6
64	0.0226	------	----	2
66	0.0156	------	----	2
83	0.0082	0.0003	3.8	4

† Results of both dynamic and static runs.

With the exception of the difference at 20 per cent relative humidity, the other information given in Table XXIV is quite similar to that previously reported. There is a significant difference between the value obtained at 61 per cent relative humidity and the value obtained at lower and higher humidities, indicating that the hostile effect of atmospheric conditions is at a maximum at or near 61 per cent relative humidity.

E. The Size and Nature of the Air-borne Particle

Measurements of the air-borne particles of S. marcescens when atomized from beef-broth extract, reported in Progress Report B-100, G-2771, C-2 (pages 32 to 34), were made very early in the investigations. The cascade-impactor values indicated an equivalent sphere of 2.0 microns in diameter, and direct measurement on settled particles with the aid of the electron microscope indicated an equivalent sphere of 2.2 microns in diameter. It was pointed out that the evidence from settling data indicated that the particle size varied (from 2.5 to 4.5 microns in diameter) directly with relative humidity.

The discrepancy between the physically measured particle size and the size determined from settling data has been considered as the only disadvantage in an otherwise good chamber operation. Because the direct measurements were probably more nearly correct, efforts were directed towards locating the possible source of error in the chamber operation. It was assumed that such an error could be due to one of two causes or to a combination of both: (1) low collection efficiency of the air samplers, giving rise to erroneous ratios between aerial concentration and numbers falling per minute per unit area and (2) some stratification within the chamber which had not been apparent in the studies on uniformity within the chamber.

Several approaches to these problems were taken. In regard to the collection efficiency of the air samplers, early data (Progress Report B-100, G-2771, C-2) indicate that the critical-orifice liquid impingers with gelatin-phosphate fluid yielded the greatest recovery of viable air-borne organisms. Since a complete evaluation of all samplers was not a primary objective of the project, published information has guided the project in the selection of a sampler and

sampler fluid. However, when some of the data obtained was unexplainable, a more thorough study of the effectiveness of air samplers was essential. The results of a study of the effect of composition of the sampler fluid on collection effectiveness showed that the use of an enriched fluid in the critical-orifice liquid impingers yielded approximately twice the numbers of viable organisms that were demonstrated from the simple gelatin-phosphate fluid. This work is given in detail elsewhere in this report. It should be emphasized that all of this work has been carried out on bacterial aerosols which are at least 90 per cent, single-bacterium-carrying particles, and that the values obtained by the method yielding the greatest number of viable organisms are likely to be the most nearly correct.

The results of the studies on the effectiveness of air samplers indicated that the combination used in much of the earlier work was approximately 50 per cent effective; the data on particle size obtained from the chamber studies were recalculated on this basis. Recalculation indicated that the probable variation range of particle size with varying relative humidity was 1.8 to about 3.0 microns in diameter instead of 2.5 to 4.5 microns which had been indicated. This range of size variation with varying relative humidity appeared to be more consistent with the earlier physical measurements of size.

The increase in the effectiveness of the air samplers yielded results which appeared to resolve the discrepancy between the physical measurements of the size of the air-borne particles and the calculated measurement from the chamber data. In addition, work also was carried out to investigate the possibility of some stratification within the chamber which had not been apparent in the original studies on uniformity of distribution of the aerosol within the chamber.

If stratification was not demonstrated by the air samples, the only probable significant error must be related to losses by fall-out or to some error in calculation of the chamber ventilation constant. Since the numbers of viable air-borne organisms demonstrated in the chamber air have been shown not to be significantly different from those numbers in the outlet air, there is little basis for believing that any unaccounted losses due to fall-out occur within the chamber. In order to determine exactly the chamber ventilation constant for aerosols, several experiments were performed on the rate of equilibration of the chamber, using the same bacterial aerosol as employed in the regular experimental work. The chamber ventilation constant for gases had been determined previously with water vapor, and the resulting value agreed with the calculated value (unity) indicating efficient operation. The results of the studies on chamber equilibration time with bacterial aerosols are shown in Chapter VI and indicate an equilibration time of slightly less than 5 minutes. This is well within the limits of error for the calculated time of equilibration using a ventilation constant of unity. Thus, for a chamber operating efficiently at one volume change per minute, a ventilation constant of unity, the time for 99 per cent equilibration is approximately 4.6 minutes. Since the chamber is 64 ft^3 in volume and is ventilated at 60 cfm, the calculated time for 99 per cent equilibration would be 4.9 minutes. It would appear, therefore, that the aerosol chamber is operating efficiently both for gases and for bacterial aerosols.

The results of the above studies seem to constitute conclusive proof that it is possible to determine accurately the size of bacterial aerosols in this chamber. A final point relating to the reliability of these measurements was verified in the course of work on the settling rate of certain bacterial

aerosols which are unusually difficult to disperse. In these studies large variations were observed among the data from the settling plates, although normal variations were observed among the data from the air samples. Careful study of this phenomenon revealed the possibility that relatively small clouds of aerosols, containing a few hundred particles bunched together, could cause the settling plate data to indicate large variations without affecting the normal variation of the air samples. This could happen because of the relative importance of the numbers involved. In the case of the settling plates, a total of not more than a few hundred particles are counted per plate, whereas, in the case of the air samples, several thousand particles are removed from the air in the process of sampling. However, it is not difficult to resolve this point because the existence of small clouds of undispersed aerosol always is manifested by the presence of extremely large variations among the settling-plate data. Conversely, the absence of such large variations among the settling-plate data apparently rules out the existence of this phenomenon.

A few experiments were carried out to verify the probable effect of impinger fluid on the calculated size of the air-borne particle. These are shown in Table XXV.

The data in this table are not entirely consistent with the conclusions of the previous discussion. At 68° F, 19 and 20 per cent relative humidity, the apparent diameter of the particles is much less than expected, either from past performance or from present considerations. However, no point in the table is derived from more than one or two runs, and it is not possible to state the significance of these data. Certainly, in general, the data in Table XXV support the conclusion that the calculation of the apparent diameter of air-borne

TABLE XXV

THE EFFECT OF CHOICE OF IMPINGER FLUID ON THE CALCULATION OF PARTICLE SIZE AS AFFECTED BY RELATIVE HUMIDITY

Temp. (°F)	Per Cent Relative Humidity	Calculated Particle Diameter From Plain-Gelatin Impinger Data (μ)	Calculated Particle Diameter From Brain-Heart Fluid Impinger Data (μ)	Ratio of Recovery of Viable Organisms Brain-Heart:Plain Gelatin
68	19	1.29	1.13	1.28
67	20	1.53	1.00	2.35
67	22	2.28	1.65	1.97
69	61	4.00	2.77	2.08
72	82	5.09	2.76	3.5
80	19	2.81	1.79	2.48
80	33	4.55	2.72	2.76
80	42	4.12	2.74	2.26
80	76	3.36	2.57	1.72

bacterial particles from data obtained using two different media will yield widely divergent results. Furthermore, the determination of particle diameter from data obtained using the more effective brain-heart collecting fluid yields values which are more nearly consistent with other measurements.

In a further attempt to verify the above information concerning the size of the air-borne bacterial particle and also to determine the physical make-up of that particle, studies were made along other lines. It was postulated that the air-borne bacterial particle under study must be formed from an initial droplet of the substrate (beef-extract-broth culture), this droplet being formed by the process of atomization. If, therefore, the total number of droplets produced

from a unit volume is known, the average volume of the individual droplet can be determined.

The studies on initial droplet size were carried out using the identical materials and equipment employed in the regular studies. The material was a routine broth culture of S. marcescens (E-R/O-ATCC-274). The equipment consisted of a DeVilbiss No. 40 atomizer, operated in the usual manner. First of all, total cell and viable counts were made, using a Petroff-Hauser cell for the total cell counts and regular dilution and plating method for the viable counts. The total cell count on the culture was 140 x 10^7/ml, and the viable count was 70 x 10^7/ml, indicating that half the cells were demonstrated as being viable.

The culture was placed in the atomizer reservoir, and the atomizer exit was connected to a 6-inch diameter settling tube. At the bottom of this tube, was placed a glass microscope slide holding electron microscope grid screens. After assembling this equipment, the atomizer air was turned on, and atomization was carried out for 1 hour. At the end of this period, the atomizer air was shut off, the assembly sealed, and allowed to stand for 6 hours to allow the air-borne particles to settle. The slide was then removed, and the grid screens examined in the electron microscope. The results of this examination are shown in Table XXVI.

The average number of bacteria per particle is estimated to be 1.61. This is to be contrasted to the earlier study on particles issuing from the prechamber where it was found that 90 per cent of these contained only single bacteria, 10 per cent contained two bacteria, and none contained more than two. This indicates the degree of effectiveness of the prechamber in preventing larger, multibacterium carrying particles from entering the main chamber.

From the above information, an estimate was made of the average size of the droplets produced by the DeVilbiss No. 40 atomizer when dispersing a beef-broth culture of S. marcescens. The average number of bacteria per particle being in excess of unity, it is reasonable to assume that all droplets produced contain at least one organism. Thus, the total number of droplets produced can be estimated from the total number of cells per milliliter of culture and the average number of cells per particle. In this case, a concentration of 140×10^7 cells per milliliter yielded particles with an average of 1.61 cells per particle or a total of 86×10^7 particles from each milliliter of culture. Because each particle arose as an initial droplet of liquid sheared off into the atmosphere by the

TABLE XXVI

NUMBERS OF BACTERIA PER PARTICLE
DISPERSED BY DeVILBISS NO. 40 ATOMIZER

Number of Bacteria Per Particle	Number of Particles Counted	Number of Bacteria Counted
1	288	288
2	78	156
3	24	72
4	6	24
5	6	30
6	8	48
7	2	14
8	5	40
Totals	417	672

action within the atomizer, it follows that this number of droplets must have been produced from each milliliter of culture. Dividing 86×10^{7} into 1 milliliter yields a value of 1170×10^{-12} milliliters for the average volume of the droplets produced under these conditions. This is equivalent to a sphere of liquid having a diameter of 13.1 microns. Taking the limiting value of 10 microns as the smallest droplet which can be produced from water by shearing atomization, and knowing that the DeVilbiss No. 40 is an efficient atomizer, the determined value of 13.1 microns is a reasonable one.

The droplet produced by atomization must start evaporating water almost immediately. Detailed and careful measurements on the bacterial cells from identical cultures showed that they are equivalent to a sphere of about 0.7 micron in diameter, having a volume of 0.18×10^{-12} milliliter. Thus, the bacterium constitutes only a small portion of the initial droplet. The beef-extract broth, therefore, is the chief constituent of the initial droplet, and the beef-extract solids would be the chief factor in determining the final equilibrium moisture content of the particle.

In order to estimate the effect of relative humidity on the moisture content of beef-extract solids, the equilibrium moisture content of beef-extract was determined at various relative humidities. This was accomplished by placing pans containing weighed amounts of beef-extract solids inside closed vessels containing saturated solutions of various salts. Each solution was chosen to yield a different vapor pressure and, therefore, a different relative humidity. The vessels were stored in the 20° C room; the samples were removed and were weighed once a week until they had ceased to gain or lose weight. The data were then assembled and plotted for interpolation. The resultant values for equilibrium moisture content of beef-extract solids are shown in Table XXVII. These values are corrected for initial moisture content

TABLE XXVII

EQUILIBRIUM MOISTURE CONTENT OF BEEF-EXTRACT SOLIDS AT VARIOUS HUMIDITIES AT 20° C

Relative Humidity (%)	Water Content (%)	Solids Content (%)
20	11	89
30	13	87
40	18	82
50	28	72
60	34	66
70	36	64
80	46	54
90	62	38
95	75	25
98	86	14

of the beef-extract solids. The beef extract used was found to yield 78 per cent solids when dried at 105° C to a constant weight.

The data in Table XXVII indicate that as relative humidity increases, the water content of the beef-extract material will increase markedly. This correlation of water content with relative humidity must, therefore, be the reason for the observed difference in particle size under varying relative humidities. If the initial droplet of 0.3 per cent beef-extract broth has a volume of 1170×10^{-12} milliliters and the initial material contains 78 per cent solids, such a droplet would contain 2.73×10^{-12} grams of anhydrous, beef-extract solids. The bacterial cell itself is only 0.18×10^{-12} milliliter in volume and can be neglected in the computation of probable particle size.

If the estimated weight of solids in the initial droplet are converted into volumes for the various relative humidities, using the equilibrium moisture data for beef-extract solids, estimates of the resultant size of the air-borne particle can be obtained. This information is shown in Table XXVIII. For purposes of comparison, the early data on particle size, as determined from chamber studies, were corrected on the basis of a 50 per cent efficiency for the impingers containing plain-gelatin sampling fluid. The original information for particle size is included in this table also.

The values shown in Table XXVIII for the calculated equivalent diameter of particles of beef-extract solids such as would result from the equilibration to various relative humidities of an initial droplet of 13.1 microns in diameter show excellent agreement with the corrected values obtained from the chamber studies. It should be pointed out that the quality of agreement may be due to coincidence because of the numerous possible errors involved in dealing with small quantities. However, the information in this table is interpreted as strong proof that the air-borne bacterial particle, as atomized from beef-extract broth, is primarily a particle of beef-extract solids. The increasing size of the air-borne particle with increasing relative humidity is, therefore, an expression of the equilibrium moisture content of the beef-extract solids. Because of the relatively smaller volume of the bacterial cell, it would be possible for that cell to vary in size under these conditions, but it would be difficult to distinguish such a change.

The results of all the above studies show conclusively that the original estimates of the size of the air-borne bacterial particles, as determined from chamber data, were in error. This error was entirely due to the failure of the

TABLE XXVIII

DATA ON PARTICLE SIZE FROM STUDIES ON AIR-BORNE PARTICLES FROM CULTURE A AT 68° F

Relative Humidity	Experimentally† Determined Equivalent Diameter	Estimated Equivalent Diameter, Gelatin Impingers Assumed 50 Per Cent Effective	Equivalent†† Diameter, Calculated From Equilibrium Moisture Content of Beef-Extract Solids, 13-μ Diameter Original Droplet
(%)	(μ)	(μ)	(μ)
20	2.6	1.83	1.80
30	2.66	1.87	1.82
40	2.8	1.97	1.84
50	2.9	2.04	1.93
60	3.0	2.12	2.00
70	3.1	2.18	2.02
80	3.25	2.29	2.13
90	3.5	2.47	2.52
95	4.2	2.96	2.76
98	---	----	3.32

† Data from Progress Report B-100 (G-2771-C2). Plain-gelatin impingers.

†† Equilibrium moisture content of beef-extract solids experimentally determined; size of initial droplet extimated from number of droplets produced from 1 ml of broth culture during atomization.

fluid (used in the impinger samplers) to collect or retain alive all of the air-borne bacterial particles (the significance of this is discussed elsewhere). The validity of the settling-plate data and of the operational characteristics of the aerosol chamber are conclusively justified.

A further result of these studies is that a definite value for the size of the initial droplet has been established, and a logical sequence for the production of the final air-borne particle has been described. The DeVilbiss No. 40 atomizer produces an initial droplet of approximately 13 microns in diameter from the beef-extract broth. These droplets contain from one to eight or more individual bacterial cells. In the prechamber, the droplets equilibrate under conditions of very high humidity, the larger droplets falling out; 90 per cent of air-borne bacterial particles which succeed in leaving the prechamber contain only one bacterium each, and the remaining 10 per cent contain only two bacteria each. The air-borne bacterial particles which enter the main chamber equilibrate to the particular humidity condition existing there. This equilibration is governed by the equilibrium moisture content of the beef-extract solids. The range of particle sizes with varying relative humidity from 20 to 90 per cent under these conditions is approximately 1.8 to 3.0 microns in diameter. It can be seen that this information could be applied readily to other substrate materials in the preparation of bacterial aerosols.

The most important result of these studies has been the establishment of the concept of the secondary environment for the air-borne bacterial cell. Various workers have assumed that the bacterium in the air was surrounded by nonliving material; much of the earlier work seemed to indicate the contrary. However, it can be concluded from the studies discussed that the air-borne

bacteria atomized from beef-extract cultures (and presumably from any fluid containing solids) exist in the air enclosed in a relatively thick coating of the solids from the substrate material. This means that the atmospheric environment is not capable of acting directly upon the air-borne bacterium but actually acts upon this coating of nonliving material, which is the true environment of the bacterium. Thus the beef-extract solids form the actual environment of the bacterium. This concept is of considerable importance in the interpretation of the results of the studies on the effect of various chemicals on air-borne organisms.

VI. STUDIES ON AERIAL DISINFECTION

The primary objective of this project was to investigate the effects of minute quantities of chemical vapors on air-borne bacteria. In the initial work program, a chamber suitable for experimental aerobiology was planned for the evaluation of aerial disinfectants. A chamber was developed, however, the full operation of this chamber arrangement requires the services of several persons (usually a minimum of two to three people), and considerable time is required in cleaning the entire system between runs made with different chemical compounds. Fortunately, in the course of work on a related project at Georgia Tech, a simplified system for experimental aerobiology was developed (Quarterly Report No. 3, Project A-155, AR & DC Project No. 22-1401-0000). This system employs the use of aerosol cylinders, described in other sections of this report. The majority of the work on the evaluation of aerial disinfectants was carried out in these cylinders rather than in the main chamber.

Several situations developed during the period covered by this report which required the expenditure of considerable personnel time and effort unanticipated in planning the program. Shortly after the period covered by the second progress report (Progress Report B-100, G-2771, C-2), the test culture underwent changes which necessitated a recharacterization and re-establishment of the original culture. More detailed discussion of the characterization and isolation of variants of the culture is presented under "Bacteriological Methods and Cultures." During the course of the studies adjunctive to the re-establishment of the culture, it was discovered that the fluid used in the aerosol samplers (plain-gelatin fluid) did not demonstrate adequately the viability of all the possible organisms. Although it was subsequently shown that the relative values for

biological die-away were essentially the same, whether determined from data resulting from use of enriched- or plain-gelatin fluid in the samplers, it was necessary to retrace many steps to prove this. The information gained from these and some minor developments proved to be quite valuable and pointed out some of the requirements pertinent to future aerobiological work.

The development of the aerosol cylinders and the realization that biological death rates can be determined with equal accuracy from either static or dynamic runs made it possible to evaluate a number of chemical compounds as aerial disinfectants. The data accumulated from these tests were sufficient to establish the quantitative relations involved in the action of aerial disinfectants. Although the main aerosol chamber was useful in the studies in experimental aerobiology and permitted the collection of certain detailed data not otherwise available, the aerosol cylinders are much simpler to use and supply sufficient information for most screening work.

The aerosol chamber and cylinders are described in some detail in other sections of this report, however, it is well to review some of the essential differences between the two pieces of equipment. Each system has distinct advantages and disadvantages, and the use of these two systems in conjunction with each other makes a complete aerobiological study possible.

The choice of which system to use depends on the type of data desired. With the main chamber, it is possible to make both dynamic and static runs, to determine the particle size of the bacterial aerosol during runs, and to evaluate the comparative efficiency of samplers. With the aerosol cylinders only the dynamic death rate may be evaluated. However, because of the higher aerial concentration that can be maintained in the cylinders, it is possible to determine

with some accuracy much greater values of k in the cylinders than can be determined in the main chamber.

The time required for the estimation of the dynamic k is about the same for both systems. However, fewer personnel are required for operating the cylinders than for operating the chamber.

From the standpoint of screening chemical compounds, the most essential difference between the systems is the time and effort required for cleaning between runs made with various chemical compounds. Almost a full day is required to clean the main chamber and its accessory equipment before the chamber is ready for use with another chemical. The cylinders were made in a series of four, and one cylinder can be easily cleaned with steam, dried with air, and be ready for use before all of the other three are used. Thus, it is possible to make several runs with the cylinders to each run made with the chamber. The only way in which the chamber can be used continuously for chemical screening is to change the operating humidity and to study the same compound under various conditions of humidity and aerial concentration. Even this recourse means that a maximum of two runs per day can be effected in the chamber. This procedure is rather unsatisfactory because of the quantities of compound required, the entire workroom becomes contaminated, and it is not possible to obtain "blank" runs.

The quantity of a compound necessary to achieve a particular concentration in the cylinders is only one-sixtieth of that required for the chamber. Although economy was not a determining factor, the problem of dispersing continuously large quantities of a compound is a difficult one. Tests made with propylene glycol showed that the nominal and actual concentrations were essentially identical for both systems. However, for other compounds, there is less danger

of loss in the cylinder system than in the main chamber system. This factor mades it simpler to rely on nominal values obtained by using the cylinders.

In considering this last point of difference between the use of the aerosol chamber and the aerosol cylinders, it is appropriate to comment on the use of the nominal concentration value for this work. A considerable amount of work has been devoted to calibrating thermal conductivity cells for estimating the concentration of various compounds in the air. In preliminary experiments, this equipment used (Gow-Mac Instrument Company, Thermal Conductivity Units) proved most useful and showed that, at low concentrations, the nominal and determined values in both systems were nearly identical. However, considerable difficulties were encountered in regular use. The difficulty, which proved insurmountable, was the fouling of the conductivity-cell wires with an aerosol of the compound under study. In practice, the compound was dispersed into the meter chamber prior to being introduced into the aerosol chamber. When the concentration of the compound in the air was high, a mist of the compound formed at the exit of the heated bubbler from which the compound was dispersed. Usually some of this mist persisted in the exit air from the meter chamber and was picked up in the thermal conductivity cell. As soon as this occurred, the conductivity measurement became useless, and several hours of purging with clean air were required before the instrument became usable again.

Because of these experiences, it was decided to employ the nominal concentration value in subsequent work, and, in all of the work reported for the cylinders, this was the case, except in the case of lactic acid. This compound dehydrates at 100° C and the nominal values showed no relation between estimated aerial concentration and rate of kill. In the work reported, the aerial concentration of lactic acid was determined by collecting an air sample in distilled

water, measuring the conductivity of the water sample (platinized electrodes and conductivity bridge), and comparing the conductivity measurement with that of known solutions of lactic acid.

Although, for the reasons given above, the majority of the work of evaluating candidate compounds as aerial disinfectants was carried out in the aerosol cylinders, certain critical experiments were accomplished in the main chamber. These related to the particle size of the bacterial aerosol in the presence of hygroscopic and nonhygroscopic compounds. Sufficient data were obtained to show the difference in the mode of action of these two different types of aerial disinfectants.

The results of the various tests carried out on a number of different compounds are given in the following pages. The results are grouped according to the equipment used for their collection, either the aerosol cylinders or the aerosol chamber.

A. Results of Chemical Screening Tests in the Aerosol Cylinders

In order to evaluate the greatest number of variables (chemical nature of compounds, fractional aerial saturation, and effect of relative humidity), the majority of the work was carried out with the aerosol cylinders, at a single condition of temperature of 68° F. (It is possible to make three to five determinations each day with the aerosol cylinders with about half the effort required to make two or three with the main aerosol chamber.) Certain critical experiments were made in the main chamber and are described elsewhere in this report.

The operational procedures involved in obtaining data with the aerosol cylinders are given in the section on equipment. The general procedure is as follows: all conditions of temperature and humidity are established in the

workroom housing the equipment; the standard bacterial aerosol (S. marcescens ATCC 274 ER) produced by the atomizer and prechamber units is established, and run through the cylinder until equilibrium is achieved; a weighed sample of the candidate compound in the chemical vapor generator (a midget impinger) is heated to 120° C; and a metered volume of dry air (usually about 500 cc/min) is bubbled through the compound and into the cylinder system at the point just prior to the mixing funnels. Vaporization of the compound is continued throughout the run. After the compound has been running into the system for 10 minutes, samples of the air in the system are taken. One sample is taken from the diluted aerosol stream at a point just prior to the entrance of the vapors of the compound, this being the control sample. Another sample is taken at the same time at the entrance to the aerosol cylinder, while a third sample is taken at the exit of the cylinder. Three sets of samples are taken for each determination. At the completion of the run, the chemical sample is removed and reweighed, and the loss per minute is calculated. Since the system is operated at 1 cfm, the loss per minute is expressed as the concentration per cubic foot. After dilution, plating, incubation, and counting, the data are reduced to terms expressing concentrations of viable organisms per liter of air. From these values, the corresponding values of the die-away, k, are calculated. The detention time in the mixing funnels is approximately 1 minute and, in the cylinder, it is 3.5 minutes; the total is 4.5 minutes. Thus, the difference between the control sample (c) and the inlet sample (in) is calculated as k_1; the difference between the inlet and outlet sample (out) is calculated as $k_{3.5}$; and the difference between the control and the outlet samples is $k_{4.5}$. Thus,

$$k_1 = \frac{\log c - \log in}{1}$$

$$k_{3.5} = \frac{\log in - \log out}{3.5}$$

$$k_{4.5} = \frac{\log c - \log out}{4.5}$$

The values for $\underline{k}_1$, $\underline{k}_{3.5}$, and $\underline{k}_{4.5}$ should be identical, within limits of experimental error and were originally planned so that the $\underline{k}_1$ and $\underline{k}_{3.5}$ values would constitute a check on the operation of the system, $\underline{k}_{4.5}$ (being essentially the average of the first two) was to be the value taken for further comparison. However, this did not prove to be the case, and all three values are reported in Table XXIX as the results of the various runs with chemicals at various concentrations and under varying conditions of relative humidity.

The differences to be noted between values for $\underline{k}_1$ and $\underline{k}_{3.5}$ for a particular set of conditions are worthy of special attention in examining the data in Table XXIX. It should be remembered that the data from which the value for $\underline{k}_1$ is calculated are obtained from samples taken just prior to and just after the chemical vapor enters the system. It is possible that slow equilibration or excessive concentration of chemical mists might be responsible for abnormal values of $\underline{k}_1$.

In a series of over 80 observations, the value of $\underline{k}_1$ is definitely lower than that for the equivalent $\underline{k}_{3.5}$ in 10 per cent of the cases. Practically all of these cases occur under conditions where the final concentration which might be achieved in the air-borne bacterial particle, would be expected to be low, because of either small fractional aerial saturation of the compound, or the reduced solubility of the compound in the particle at low relative humidity.

TABLE XXIX

RESULTS OF CHEMICAL SCREENING TESTS IN AEROSOL CYLINDERS AT 68° F

Chemical Compound	Per Cent Relative Humidity	Per Cent Aerial Saturation (Dry Air Basis)	Die-Away Values k_1	$k_{3.5}$	$k_{4.5}$
2- Ethylhexanediol (1,3)	25	100	0.00	0.035	0.015
	25	100	0.027	0.033	0.033
	30	28	0.00	0.033	0.014
	30	150	0.027	0.078	0.067
	50	120	0.023	0.19	0.16
	58	120	0.38	0.19	0.24
	62	100	0.24	0.32	0.29
	65	54	0.11	0.04	0.057
	65	84	0.16	0.19	0.18
	65	95	0.13	0.21	0.19
	80	22	0.039	0.039	0.039
	80	32	0.046	0.040	0.041
	80	36	0.072	0.057	0.060
	80	48	0.085	0.185	0.163
	80	88	0.192	0.28	0.242
	80	92	0.208	0.21	0.21
	80	120	0.36	0.28	0.30
	80	125	0.21	0.29	0.27
Triethylene Glycol	25	1000	0.17	0.20	0.19
	25	1000	0.14	0.23	0.21
	50	120	0.15	0.05	0.072
	80	40	0.11	0.041	0.056

(Continued)

TABLE XXIX (Continued)
RESULTS OF CHEMICAL SCREENING TESTS IN AEROSOL CYLINDERS AT 68° F

Chemical Compound	Per Cent Relative Humidity	Per Cent Aerial Saturation (Dry Air Basis)	Die-Away Values		
			k_1	$k_{3.5}$	$k_{4.5}$
Propylene Glycol	25	100	0.23	0.23	0.23
	50	40	0.156	0.064	0.09
	50	80	0.25	0.10	0.135
	50	100	0.51	0.57	0.56
	50	140	2.8	0.15	0.74
	70	110	0.096	0.042	0.067
Diethylene glycol-mono-butyl ether	25	400	0.40	0.22	0.26
	30	120	0.28	0.15	0.18
	30	20	0.12	0.078	0.087
	50	40	0.55	0.178	0.26
	50	100	0.44	0.18	0.23
	50	120	1.09	0.43	0.60
	50	140	0.55	0.24	0.31
	50	200	2.0	0.55	0.87
	60	140	0.63	0.26	0.34
	65	50	0.5	0.25	0.31
	68	35	0.65	0.26	0.35
	80	200	2.2	>1	>1
	90	72	1.5	0.33	0.61
Propanediol (1,3)	25	100	0.20	0.22	0.22
	50	93	0.19	0.19	0.19
	80	26	0.16	0.05	0.07
Hexanediol (2,5)	25	120	0.166	0.14	0.15
	50	80	0.33	0.15	0.19
	80	120	0.20	0.37	0.33

(Continued)

TABLE XXIX (Continued)

RESULTS OF CHEMICAL SCREENING TESTS IN AEROSOL CYLINDERS AT 68° F

Chemical Compound	Per Cent Relative Humidity	Per Cent Aerial Saturation (Dry Air Basis)	Die-Away Values k_1	$k_{3.5}$	$k_{4.5}$
2-Ethylhexanol	25	600	0.043	0.018	0.023
	50	130	-----	0.048	0.037
	80	170	0.156	0.11	0.12
Ethylene glycol-mono-butyl ether	25	100	0.11	0.12	0.12
	50	31	0.13	0.12	0.12
	80	43	0.32	0.18	0.21
Hexanedione (2,5)	25	100	0.11	0.05	0.062
	50	30	0.10	0.053	0.064
	80	23	0.04	0.065	0.06
Octanol (1)	25	50	0.078	0.017	0.031
	50	90	0.20	0.095	0.12
	80	90	0.30	0.20	0.22
Ethylene Glycol	50	90	-----	0.031	0.024
	80	20	0.13	0.031	0.053
Lactic Acid	50	100+	1.8	0.41	0.72
	50	100+	1.9	0.53	0.85
	80	10	0.18	0.00	0.036
	80	27	0.89	0.185	0.34
Dimethyl Phthalate	25	400	0.056	0.017	0.026
	50	400	0.000	0.061	0.048
	80	400	0.044	0.064	0.059
Diethyl Phthalate	50	300	0.005	0.030	0.023
	80	300	0.018	0.047	0.040
Dibutyl Phthalate	50	400	0.10	0.035	0.052

(Continued)

TABLE XXIX (Continued)

RESULTS OF CHEMICAL SCREENING TESTS IN AEROSOL CYLINDERS AT 68° F

Chemical Compound	Per Cent Relative Humidity	Per Cent Aerial Saturation (Dry Air Basis)	Die-Away Values		
			k_1	$k_{3.5}$	$k_{4.5}$
Diethylene glycol-mono-ethyl ether	80	15	0.12	0.19	0.16
Thiodiglycol	25	100	0.013	0.12	0.094
	50	100	0.042	0.041	0.041
	50	100+	0.28	0.081	0.12
n-Butyl Lactate	50	100	0.12	0.19	0.17
	80	100	0.19	0.09	0.11
Triethylenetetramine	50	72	0.31	0.034	0.095
	80	35	0.29	0.000	0.064
Nitrobenzene	50	10	0.013	0.041	0.035
Water	50	95	0.014	0.018	0.017
	50	135	0.11	0.022	0.042

Out of this same series, approximately 20 per cent of the recorded values for k_1 shown in Table XXIX are higher than the corresponding value for $k_{3.5}$. Practically all of these cases occur under conditions of saturation or excess of saturation concentrations of the chemical in the air. It is thus likely that this difference is caused by the existence of higher concentrations of compound at the exit of the chemical-vapor generator.

The remaining 70 per cent of the values for k_1 shown in Table XXIX are essentially identical with the corresponding value of $k_{3.5}$. In this connection, attention is called to the last two sets of values for water vapor in Table XXIX. In these cases, the system was operating at 50 per cent relative humidity,

and the chemical-vapor generator was loaded with water in the usual manner but was heated only to 90° C. The resulting values of 95 and 135 per cent of aerial saturation were obtained. In the case of the 95 per cent saturation, $\underline{k}_1$ is equal to $\underline{k}_{3.5}$, but, at 135 per cent saturation, $\underline{k}_1$ is definitely larger than $\underline{k}_{3.5}$. In light of the explanations offered above, this information seems to describe the situation adequately. The values for $\underline{k}_1$ and $\underline{k}_{3.5}$ should be essentially the same, but excessive quantities of chemical can cause an abnormally high value of $\underline{k}_1$ to be obtained. No satisfactory explanation for this phenomenon has been found.

The bulk of the information in Table XXIX is self-evident. For convenience, the essential data from this table have been compiled, along with certain physical properties of the compounds, and are shown in Table XXX. A detailed analysis of the significance of this information is in section entitled "Mode of Action of Nonhygroscopic Compounds as Aerial Disinfectants." However, a brief study of the data in Table XXX shows that no single one of these compounds embodies the characteristics of the ideal aerial bactericide. The hygroscopic compounds which exhibit their maximum killing power as aerial disinfectants at 25 per cent relative humidity possess little activity at 80 per cent relative humidity; the converse is true of the nonhygroscopic compounds. These observations are representative of the numerous reports in the literature. Although this present search has not revealed the ideal aerial germicide, the methods outlined in this report should be helpful in facilitating further search for such a compound.

TABLE XXX

PHYSICAL PROPERTIES OF CERTAIN CHEMICAL COMPOUNDS AND THEIR ESTIMATED† MAXIMUM KILLING POWER AS AERIAL DISINFECTANTS

Chemical Compound	Vapor Pressure, mm Hg at 20° C	Approximate Aerial Saturation, mg/ft^3 of Dry Air	Solubility†† in water, gm/100 gm	Estimated Maximum k at Aerial Saturation	
				At 25 per cent RH	At 80 per cent RH
2-ethylhexanediol (1,3)	0.011	2.5	4.0	0.01	0.30
Triethylene glycol	0.001	0.25	M-H	0.30	0.01
Propylene glycol	0.08	9.5	M-H	0.25	0.01
Diethylene glycol-mono-butyl ether	0.023	5.5	M-N	0.2	0.7
Propanediol (1,3)	0.057	6.5	M-H	0.2	0.05
Hexanediol (2,5)	0.02	3.5	M-N	0.15	0.4
2-ethylhexanol	0.05	10	0.1	0.02	0.1
Ethylene glycol-mono-butyl ether	0.76	140	M-N	0.1	0.3
Hexanedione (2,5)	0.43	76	M-N	0.05	0.7
Octanol (1)	0.08	16	0.05	0.01	0.2
Ethylene glycol	0.05	6	M-H	0.1	0.05
Lactic Acid	0.004	0.55	M-H	ca 1	0.2

(Continued)

TABLE XXX (Continued)

PHYSICAL PROPERTIES OF CERTAIN CHEMICAL COMPOUNDS AND THEIR ESTIMATED† MAXIMUM KILLING POWER AS AERIAL DISINFECTANTS

Chemical Compound	Vapor Pressure, mm Hg at 20° C	Approximate Aerial Saturation, mg/ft^3 of Dry Air	Solubility†† in water, gm/100 gm	Estimated Maximum k at Aerial Saturation	
				At 25 per cent RH	At 80 per cent RH
Dimethyl Phthalate	0.004	1.3	0.5	0.01	0.06
Diethyl Phthalate	0.003	0.9	---	0.01	0.04
Dibutyl Phthalate	0.0005	0.2	0.04	0.01	0.04
Diethylene glycol-monoethyl ether	0.18	37	M-N	----	0.8
Thiodiglycol	0.01	1.9	M-N	0.05	0.1

† Either taken directly from chemical studies or calculated from equations under "Mode of Action of Nonhygroscopic Aerial Disinfectants," or from section on Puck's Equation.

†† The symbols used in this column are as follows: M = miscible with water in all proportions; H = hygroscopic; N = nonhygroscopic.

B. Results of Studies in the Main Aerosol Chamber

As indicated in the introduction to this section, the majority of the studies on aerial disinfection were carried out in the aerosol cylinders in order to save time and labor. However, a sufficient amount of detailed work on the action of chemicals as aerial disinfectants was carried out in the chamber to indicate the differences between the action of hygroscopic and nonhygroscopic compounds. Propylene glycol was selected as representative of the hygroscopic chemical compounds showing effectiveness as aerial disinfectants. This compound was chosen because previous studies using the thermal conductivity cell to measure concentration, showed that desired concentrations of the compound could be maintained in the chamber under dynamic conditions and because it could be dispersed in varying quantities. During the course of the screening work, another glycol which was nonhygroscopic and of limited water solubility (approximately 4 grams per 100 grams of water) was found to show activity as an aerial disinfectant approximately equal to that of propylene glycol (although not under the same conditions of humidity). Therefore, this compound, 2-ethylhexanediol (1,3), was selected for these tests as representative of the nonhygroscopic compounds which act as aerial disinfectants.

The desire to compare in detail the action of hygroscopic and nonhygroscopic compounds was based on the assumption that the former compounds would cause a definite enlargement of the bacterial particle under certain conditions of relative humidity, whereas it was predicted that the latter would not do so. It was further desired to determine whether propylene glycol acted as an aerial disinfectant by killing of air-borne bacteria or by the mechanism of enlargement and sedimentation of the bacterial particle.

In the experiments reported here, the majority of the information for the aerial concentration was determined from nominal concentrations, i. e., the compound was dispersed from a weighed, heated bubbler (250-ml capacity), and a stream of dry air was injected through it during the course of the run. At the end of the run, the bubbler was reweighed, and the aerial concentration was determined from the weight loss and the time lapse of the run. This value was reported as the nominal concentration. The per cent aerial saturation was also calculated on a dry air basis from vapor pressure data.

Each particular run consisted of a dynamic and a static study, using the standard aerosol of S. marcescens (ATCC ER) and previously described equipment and methods. During the dynamic study, air samples were withdrawn from the inlet, chamber, and outlet (3 samples each, procedure repeated 3 times). Agar settling plates were exposed simultaneously at the bottom of the chamber (12 to 24 plates per run). During the static study, settling plates were exposed for 1 minute at 1-minute intervals for a total of 30 minutes. The dynamic k_t was calculated from the inlet and outlet aerosol concentration data. The apparent particle diameter was calculated by comparing the numbers falling per minute during the dynamic study to the aerial concentration of organisms during the same study. The value for k_f was also determined from this information and was subtracted from the k_t value to yield the net k due to death. For purpose of comparison, the killing effected by the relative humidity was not subtracted. It will be noted that these values for the effect of relative humidity are greater than those reported elsewhere, probably due to contamination of the workroom air during these runs. For the majority of the runs, the k_t values were so great that the results from the static studies were of little value;

the high rate of disappearance of the organisms resulted in sterile plates in too short a time period to allow the calculation of the settling rate from these results. The static studies which were made at those conditions yielding low values of $\underline{k}_t$ as determined dynamically also showed comparable values of $\underline{k}_t$ statically. However, these were so few in number that they are not reported here.

The results of the studies with propylene glycol are given in Table XXXI. The values given for $\underline{k}_t$ represent the total action of the compound in removing bacteria from the air, both by killing and by sedimentation. The values shown in Table XXXI indicate that, at high concentrations of the compound in the air, a definite activity exists at all humidity conditions. However, the information given for the apparent particle diameter shows a distinct difference in response under the varying humidity conditions; as the relative humidity is increased, the particle size increased markedly. It should be pointed out that the indicated particle size is the minimum value. No studies were made with especially enriched agar plates to determine whether any greater number could be demonstrated. If the bacterial particle increased in size as much as indicated, then it must have been carrying a heavy load of water and propylene glycol. Such a bacterial particle might well contain an "insulted" organism which could be demonstrated as viable if properly collected. Any increase in the number of of viables collected on the settling plates would have resulted in an increase in the apparent particle diameter.

The last column in Table XXXI gives the data for the net $\underline{k}$ due to death. Here the sedimentation effect has been deleted from the $\underline{k}_t$ resulting in a lowered value at the higher humidity. However, the air saturated with propylene glycol at 85 per cent relative humidity still exhibits a definite activity as an

aerial bactericide. This is contrary to the data obtained from the aerosol cylinders at 70 per cent relative humidity, where a much lower activity is shown. It should be noted that the data from the aerosol cylinders are obtained from air samples only, whereas the data from the chamber are obtained from both air and settling samples. It is suggested that the possibility proposed above concerning "insulted" organisms might explain the difference between the chamber data and cylinder data in this particular case.

In general, the data in Table XXXI show that propylene glycol exhibits a definite activity as an aerial disinfectant. At humidities above 45 per cent, a considerable portion of this activity is due to enlargement and sedimentation of the air-borne bacterial particles, although a definite activity as an aerial bactericide also is shown under these conditions (it must be remembered that these tests were carried out on essentially single-bacterium aerosol particles). The maximum size indicated in Table XXXI in the absence of propylene glycol is 3.0 microns. If the tests had been carried out with larger initial particles, the effect of enlargement and sedimentation by propylene glycol would have been much more evident. In the present instance, the enlargement of a 3.0-micron particle to approximately 9 microns involves a 27-fold increase in volume. Under the same conditions, a particle initially 6 microns in diameter would have enlarged to 18 microns, and the k_f would be approximately 0.2. In such a case, no bactericidal action would have been exhibited at the higher relative humidities where the particles showed definite increases in size. Furthermore, any studies carried out in ducts, where the height would be appreciably less than that of the 4-foot cubical chamber, would also show a greatly increased fall-out factor under conditions of high relative humidity.

Interpreting the data shown in Table XXXI in the light of the above observations, it seems likely that most of the published information on the action of propylene glycol can be resolved. Those workers who have studied larger bacterial aerosol particles or who have carried out experiments in ducts have indicated that the action of this compound as an aerial disinfectant is primarily due to enlargement and sedimentation of the bacterial aerosol. Those who have carried out experiments with small air-borne bacterial particles in larger chambers have reported a definite aerial bactericidal effect. Properly interpreted, no conflict exists between the results obtained by these various workers. However, there is one point of conflict regarding the fate of the sedimented organisms under these conditions. It has been suggested that such organisms when subsequently dehydrated might be resuspended and reinfect the air. The marked and definite bactericidal activity of propylene glycol at the lower relative humidities would make this impossible. In the process of drying, the lethal action of the propylene glycol would be manifested and the organisms would die.

The results of the studies made with the nonhygroscopic compound, 2-ethyl-hexanediol (1,3) are shown in Table XXXII. It can be seen that, even at 85 per cent relative humidity, there is only a slight increase in the apparent particle size, although this might represent as much as a twofold increase in particle volume. However, this increase is insufficient to cause fall-out to play any significant part in the aerial disinfectant activity of this compound. In agreement with the data obtained from the aerosol cylinders, the killing power of this compound increases with increasing relative humidity. This is the reverse of the situation with the hygroscopic compounds and verifies the

TABLE XXXI

RESULTS OF CHAMBER STUDIES OF THE ACTION OF PROPYLENE GLYCOL ON AIR-BORNE BACTERIA AT 68° F

Per Cent Relative Humidity	Aerial Concentration of Compound (mg/ft^3)	Per Cent Aerial Saturation (Dry Air Basis)	Dynamic k_t	Apparent Particle Diameter, Microns†	Net $\underline{k}$ Due to Death
40	15.8	70	0.28	2.8	0.27
45	8.1	85	0.32	3.1	0.31
45	0.0	0	0.090	2.3	0.085
55	8.2	86	0.28	7.9	0.25
55	4.9	52	0.28	8.6	0.23
55	1.6	17	0.048	2.5	0.041
55	0.0	0	0.032	2.4	0.026
85	1.0	11	0.053	4.0	0.043
85	9.5	100	0.22	9.4	0.16
85	0.0	0	0.040	3.0	0.034

† Calculated from the aerial concentration of viable organisms and the numbers of viable organisms settling per minute during the dynamic runs.

original assumption regarding the difference in the mode of action of these two classes of aerial disinfectants.

In general, the results obtained from the use of both the aerosol chamber and the aerosol cylinders indicate that the hygroscopic compounds which act as aerial disinfectants show definite activity at almost all conditions of relative humidity. At lower humidities, this activity is primarily due to bactericidal

TABLE XXXII

RESULTS OF CHAMBER STUDIES OF THE ACTION OF 2-ETHYLHEXANEDIOL (1,3) ON AIR-BORNE BACTERIA AT 68° F

Per Cent Relative Humidity	Aerial Concentration of Compound $(mg/ft)^3$	Per Cent Aerial Saturation (Dry Air Basis)	Dynamic k_t	Apparent Particle Diameter, Microns†	Net $\underline{k}$ Due to Death
60	1.66	66	0.13	2.7	0.12
60	3.66	145	0.18	2.9	0.17
60	9.2	370	0.21	2.8	0.20
60	0.0	0	0.07	2.6	0.06
85	1.66	66	0.14	4.0	0.13
85	3.3	135	0.40	4.1	0.39
85	0.0	0	0.04	3.0	0.034

† Calculated from the aerial concentration of viable organisms and the numbers of viable organisms settling per minute during the dynamic runs.

action, and, at higher humidities, it is partly or entirely due to sedimentation of the bacterial particles (depending upon the original size of the air-borne bacterial particle). On the other hand, it is indicated that nonhygroscopic compounds which show activity as aerial disinfectants do so almost entirely through their power as bactericides, and that they exhibit this power increasingly with increasing relative humidity.

VII. THE MODE OF ACTION OF AERIAL DISINFECTANTS

A. The Mode of Action of Nonhygroscopic Aerial Disinfectants

In deriving concepts for the formulation of equations to describe the mode of action of aerial bactericides, preliminary studies were made with hygroscopic materials, using triethylene glycol (TEG) as a model compound. The results of these studies are detailed in the appendix. It was found that certain of the original postulates had to be modified as a result of this study, and the knowledge so gained has been applied also to the formulation of equations to describe the mode of action of nonhygroscopic aerial disinfectants having a vapor pressure less than that of water.

1. Postulates

The following underlying postulates are made concerning the mode of action of both hygroscopic and nonhygroscopic aerial disinfectants.

a. Bactericidal Activity of a Chemical. The primary bactericidal activity of a chemical is the same whether the bacteria are suspended *in vitro* or in the air.

b. Environment of the Bacteria. Bacteria when air-borne are ordinarily surrounded by a layer of nonliving material which makes up the immediate environment of the organism; when suspended *in vitro* the organism is in more direct contact with the larger environment.

c. Rates of Bacterial Kill. Equal rates of bacterial kill will result from equal fractions of saturation of the chemical either *in vitro* or in the air-borne bacterial particle. For water-miscible compounds, per cent of saturation and per cent concentration are synonymous.

d. Fractional Saturation of the Chemical in the Air-borne Particle. The fraction of saturation of the chemical achieved in the air-borne particle as a result of a particular aerial concentration of a compound is not necessarily the same as the fractional saturation of the chemical in the air. There are three reasons why this is true.

(1) Relative humidity has a most important modifying effect upon the maximum fractional saturation achieved in the air-borne particle. In the case of hygroscopic chemicals, relative humidity determines the concentration of the chemical in the air-borne particles in terms of humectant concentrations; in the case of nonhygroscopic chemicals, the equilibrium moisture content of the air-borne particle (determined by the relative humidity) modifies the solubility of the compound in the particle.

(2) In general, for small fractions of saturation of the chemical in the air, the resultant fraction of saturation in the air-borne particle will be greater than expected. Strong driving forces directed toward the achievement of humectant concentrations for hygroscopic materials and of saturation for nonhygroscopic materials apparently exist.

(3) Under conditions of aerial saturation of the compound, the condition of humectant concentration or of saturation will be approached but not completely achieved because of the high solids content of the nonliving material surrounding the air-borne bacteria.

e. Mechanism of Transfer of Compound to the Air-borne Particle. In all this work, it is assumed that the mechanism of transfer of the compound from the gaseous state to the air-borne particle is accomplished by condensation and solution. Published information seems adequate to warrant this assumption.

2. Experimental Work

The above postulates did not originate with the project. They are the synthesis of the conclusions of other workers, modified according to our own investigations. It was felt that the intense interest in hygroscopic compounds exhibited by so many workers was stifling interest in researches directed at the mode of action of aerial disinfectants in general. A great deal of time was devoted to searching for a nonhygroscopic compound having a sufficient number of the desired characteristics to serve as a study model. Compounds not employed previously as an aerial disinfectant were evaluated. The compound 2-ethylhexanediol (1,3) was found to be satisfactory. This compound was obtained through normal channels from a laboratory supply house. It is closely related to TEG, being a diol, more commonly called a glycol. The physical data presented in Table XXXIII were obtained from the information sheets of Carbide and Carbon Chemical Company.

TABLE XXXIII

PHYSICAL PROPERTIES OF 2-ETHYLHEXANEDIOL (1,3)

Molecular Weight	Specific Gravity	Boiling Point (°C)	Vapor Pressure mm Hg 20° C	Solidification Point (°C)	Solubility Per Cent by Weight at 20° C In Water	Water In
146.22	0.9422	243.1	< 0.01	-40	4.2	11.7

The vapor pressure, as estimated from the data given by Stull† using a Cox chart, is 0.011 mm of mercury at 20° C. This yields a saturation value of approximately 2.5 mg/ft^3 for air at 68° F (20° C). The solubility in water was found to be

† Stull, I. E. C. 36, 517 (1947).

nearly 4.0 per cent. As can be seen, the only radical difference in physical properties between 2-ethylhexanediol (1,3) and TEG is that the latter is completely miscible with water and is hygroscopic.

The data essential to the support or denial of the postulates given were collected using 2-ethylhexanediol (1,3). These data consist of the in vitro killing power of the compound, the variation of the aerial killing power with variation in aerial concentration of the compound, and the effect of relative humidity on the aerial killing power of the compound. These data are shown in Tables XXXIV through XXXVI, respectively. The nature of the nonliving material making up the immediate environment of the air-borne bacterium was determined previously. This is discussed elsewhere, but, for the sake of completeness, data are shown in Table XXXVII for the equilibrium moisture content of beef extract (used in this work) and of saliva (Studies In Air Hygiene, Medical Research Council Special Report Series No. 262, H. 1M. Stationery Office, London, England, 1948, p 107).

The most important conclusion to be drawn from a study of Tables XXXIV through XXXVI can be inferred from Table XXXVI, which shows the variation in aerial killing rate of 2-ethylhexanediol (1,3) caused by relative humidity (the aerial concentration of the compound always being at or above saturation). As the relative humidity increases, the killing rate increases, up to about 60 per cent humidity and then levels off. This is the opposite of the effect of relative humidity on the aerial killing power of the hygroscopic TEG (see appendix). In this case, from a maximum at about 30 per cent relative humidity, the killing rate steadily declines with increasing humidity, which is predicted under VII-A-1d(1). The concentration of the hygroscopic compound is determined by the humectant

TABLE XXXIV

IN VITRO TOXICITY OF 2-ETHYLHEXANEDIOL (1,3)†

Aqueous Concentration, Per Cent	Per Cent Saturation In Water	k
1.2	30	< 0.02
2.0	50	< 0.02
2.8	70	0.155
3.2	80	0.20
3.6	90	0.6

† Approximation equation:

$$\log(100k) = 6.2(\log F) - 10.25$$

where F is per cent fractional saturation of compound in water.

concentration, which is progressively lower as the relative humidity increases. The concentration of the nonhygroscopic material increases as the solids content of the immediate environment of the air-borne bacteria is decreased by increasing humidity (see Table XXXVII).

Comparison of the data from the original references to the discussion in the section of this report on Puck's equation will show that the in vitro toxicity and the general values for aerial toxicity at various levels of fractional aerial saturation are essentially the same for TEG and 2-ethylhexanediol (1,3), although TEG yields higher maximum killing rates at concentrations approaching 100 per cent.

3. In Vitro Toxicity

Reference to Tables XXXIV to XXXVI will show that an approximation equation is given for each. These equations are merely descriptive of the data

TABLE XXXV

AERIAL TOXICITY OF 2-ETHYLHEXANEDIOL (1,3) AT 80 PER CENT RELATIVE HUMIDITY (68° F)†

Nominal Aerial Concentration (mg/ft^3)	Fractional Saturation in Air (%)	Net $k_{3.5}$
0.55	22	0.019
0.65	32	0.020
0.90	36	0.037
1.2	48	0.165
2.2	88	0.19
2.5	100	0.26
3.0	100+	0.21
3.2	100+	0.26
3.4	100+	0.27

† Approximation equation:

$$\log F_A + 0.8(2 - \log F_A) = \log C_{RH}$$

where F_A = per cent saturation in the air,

C_{RH} = a factor to be applied to the maximum equivalent fractional saturation achievable at any relative humidity.

in these tables. Attempts to equate aerial killing power in theoretical terms by this project and by other workers in the past have proven unsuccessful. It is concluded that this failure is due to the fact that a theoretical equation must include all of the factors relating to chemical toxicity toward living cells. This would include chemical activity, cell interface activity, strategic enzyme activities, general condition of the cell, and other variables.

These have not yet been equated for the more simple case of *in vitro* killing power, despite the volumes written on the subject. The only reasonable approach to a description of the mode of action of aerial disinfectants is that given in the first postulate--that the general mechanism of chemical activity against the living cell is the same for either *in vitro* or aerial toxicity. If it is assumed that *in vitro* toxicity and aerial toxicity are basically the same, the

TABLE XXXVI

EFFECT OF RELATIVE HUMIDITY ON THE DEATH RATE OF AIR-BORNE BACTERIA CAUSED BY AIR SATURATED WITH 2-ETHYLHEXANEDIOL (1,3) AT 68° F†

Per Cent Relative Humidity (%)	Aerial Concentration of Compound (mg/ft^3)	Net $k_{3.5}$
25	2.5	0.01
30	8.3	0.06
50	3.4	0.17
58	3.4	0.17
62	2.6	0.30
80	2.5	0.26
90	4.2	0.25
92	3.3	0.28

† Approximation equation:

$$\log RH + 0.8(2 - \log RH) - 0.079 = \log F_{PM}$$

where RH = relative humidity expressed in per cent, and
F_{PM} = the maximum per cent of saturation that can be achieved in the air-borne particle at the particular relative humidity, the air being saturated with the chemical.

problem then becomes one of equating the two conditions.

The approximation equation for in vitro toxicity of 2-ethylhexanediol (1,3) against S. marcescens (ATCC 274 ER) given in Table XXXIV is

$$\log (100\ k) = 6.2\ (\log F) - 10.25 \qquad (1)$$

where k = biological die-away, and

F = per cent aqueous saturation of the compound

TABLE XXXVII

EQUILIBRIUM MOISTURE CONTENT OF THE IMMEDIATE ENVIRONMENT OF AIR-BORNE BACTERIA

Per Cent, Relative Humidity	Equilibrium Moisture Content of Material Surrounding Air-Borne Bacteria, in Per Cent Water	
	Beef Extract, When Bacteria is Atomized From Broth	Saliva, When Bacteria is Atomized by "Spraying Spit"†
25	11	----
30	13	7.6
40	18	14.1
50	29	24.7
60	33	37.1
70	36	51.0
80	46	65.0
90	62	78.0

† From Studies in Air Hygiene, Medical Research Council Special Report Series No. 262, H. M. Stationery Office, London, England, 1948.

The value of k in equation 1 is a common type of expression for the biological die-away, being first expressed by Harriette Chick,† and the exponential die-away indicated by k is known as Chick's Law. As a point of emphasis as to the enormity of the task involved in presently equating all the factors involved in aerial killing of bacteria, it should be noted that no satisfactory explanation is yet available for this commonly noted phenomenon. Equation 1 is modeled after the expression used by R. C. Jordan†† in studies on the dynamics of *in vitro* disinfection. It is especially noteworthy that in Jordan's equation dealing with the killing power of phenol, the slope of the line is 6.9638 for 99 per cent kill. In the present case, the slope is 6.2 for 90 per cent kill with an entirely different compound. The slope of the similar line for TEG is 6.15. A literature search also revealed that, for compounds having a high vapor pressure, the slope of the line of killing power versus concentration (or fractional saturation) varies from 1 to 2, for either vegetative forms or spores; for chlorine against vegetative forms,††† for chlorine versus spores,†††† and for ethylene oxide versus spores.†††††

A discussion of the primary mechanics of toxicity is not intended here; however, if *in vitro* toxicity data are to be equated to aerial toxicity, a

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† Chick, Harriette, J. Hyg. 8, 92 (1908).

†† Jordan, R. C., J. Hyg. 43, 363 (1944).

††† Fair, G. M., et al., J. Am. W. W. A. 40, 1051 (1948).

†††† Charlton, D., and Levine, M., A & M E. E. S. Bull. No. 132 (1937).

††††† Kaye, S., and Phillip, C. R., Am. J. Hyg. 50, 296 (1949).

discussion of the various methods of expressing results is in order. The biological die-away, k, is extremely useful in expressing the results of aerial studies. However, because of the considerable amount of work involved in obtaining exact k values for in vitro work, a number of other methods of expression are used for this purpose. Some of the relations are self-evident but are indicated here for purposes of comparison. For example, the time required for 90 per cent kill (t_{90}) is 1/k; for 99 per cent kill (t_{99}) is 2/k; and for 99.9 per cent kill ($t_{99.9}$) is 3/k. The virtual sterilization time (vst) is generally taken as the time for 99.9 per cent kill. The phenol coefficient is that dilution of the compound in water which will kill a test organism in 10 minutes but not in 5 minutes, phenol being the standard of reference. As a rough estimate, it would seem reasonable to say that this is equal to $t_{99.9}$ of 10 minutes for the dilution stated by the phenol coefficient. Other methods of expressing germicidal action in terms of time required to effect per cent kill are readily converted to k, for the particular concentration involved. At the present time, there is insufficient knowledge of the action of various germicides to be certain that all compounds of low vapor pressure can be equated in terms of concentration (or fractional saturation) versus killing power with a slope of approximately 6, or that compounds with high vapor pressures will similarly yield a slope varying from 1 to 2. However, in screening compounds for candidate aerial germicides, these rough approximations will be of value, because a considerable amount of data of this type have been published for a wide variety of compounds.

4. The Relationship Between Aerial Concentration and Killing Power of the Chemical

In Table XXXV data are presented showing the various values of k obtained from studies using various concentrations of 2-ethylhexanediol (1,3) in the air

at 80 per cent relative humidity and 68° F. This relative humidity was chosen because it appeared to be one at which this compound exhibited its greatest killing power. It is to be noted that, in Table XXXV, the aerial concentration of the compound is given in mg/ft^3 and also in per cent fractional saturation in the air. It was suggested by Ferguson† that the bactericidal activity of a homologous series could be related, on a gram-molecular-weight basis, as a ratio of minimum bactericidal concentration to the saturation concentration. In comparing various compounds, this concept is most readily expressed in terms of fractional saturation, and, in the work herein reported, this has been found to be most useful.

The approximation equation describing the relationship between per cent fractional saturation in the air and killing power, as stated in Table XXXV, is expressed in terms of a constant, C_{RH}. This constant is applied to the maximum equivalent fractional saturation which can be achieved at a given relative humidity. The equation was derived by relating the aerial killing rate data to the in vitro killing rate data. This follows from the third postulate (that equal rates of bacterial kill will result from equal fractions of saturation of the chemical either in vitro or in the air-borne bacterial particle). The equation is as follows:

$$\log C_{RH} = (F_A) + 0.8\ (2 - \log F_A) \qquad (2)$$

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† Ferguson, Proc. Roy. Soc. 127, Series B, 387 (1939).

where F_A = per cent aerial saturation of the compound, and

C_{RH} = per cent of maximum saturation achieved in the air-borne particle under the conditions of the stated fractional aerial saturation.

Because relative humidity is a limiting factor in the equivalent fractional saturation achieved in an air-borne particle, C_{RH} is considered simply as a constant to be applied to the maximum values obtainable at the specified relative humidity.

The data in Table XXXV and equation 2 which describes these data, indicate that the fraction of saturation achieved in the air-borne particle for conditions of low fractional aerial saturation is relatively greater than might be expected. This is the same phenomenon as encountered with TEG (see appendix). In the case of the hygroscopic compound, this was not unexpected, as it had been predicted that the humectant action of the compound would constitute a driving force. That is, the tendency would be for any free water to attract the hygroscopic vapors and, by condensation and solution, tend to build up to humectant concentration in the water. Because TEG has a vapor pressure much less than water, there would be no driving force towards evaporation until humectant concentration was achieved, and, at aerial concentrations below saturation, this condition might not exist for a long time. After studying the available data for nonhygroscopic compounds, it was concluded that a similar set of conditions must exist for any water-soluble compound having a low vapor pressure. Even at small fractions of aerial saturation, there must be some condensation and solution in free water, and there is no driving force to evaporate the compound, once it is dissolved. Such a course of events would cause the build-up of larger fractions of saturation in the water than exist in the surrounding air.

This observation should be of some value in formulating useful conditions for a practical aerial disinfectant. For example, if the compound exerts marked killing power at a low fractional saturation in water, a comparable killing power in the air should result from an even lower fractional aerial saturation.

5. The Relationship Between Killing Power and Relative Humidity

In Table XXXVI are presented data for the effect of relative humidity on the aerial killing power of 2-ethylhexanediol (1,3) at conditions of aerial saturation or greater. The approximation equation for these data is

$$\log F_{PM} = \log RH + 0.8\ (2 - \log RH) - 0.079 \qquad (3)$$

where F_{PM} = maximum per cent saturation that can be achieved in the air-borne particle at the stated relative humidity, the air being saturated with the compound, and

RH = per cent relative humidity.

This relationship was determined by assuming, as previously stated, that equal fractions of saturation of a compound *in vitro* and in the air-borne particle would result in equal killing rates. The values for k obtained at the various conditions of relative humidity were compared with the *in vitro* data given in Table XXXIV and the fractional saturation in the air-borne particle determined on the basis of equal fractional saturation for equal k values. The value 0.079 in equation 3 is merely an expression of the observed fact that equivalent aqueous saturation of 100 per cent is approached but never achieved in the air-borne particles. This is presumably due to the solids content of the particle.

In analyzing the data in Table XXXVI it was found that the primary relationship between fractional saturation achieved in the air-borne particle and the

relative humidity is actually in terms of the concentration of water in the air-borne particle (see Table XXXVII). However, the relative humidity is the factor determining the equilibrium moisture concentration in the air-borne particle (in the absence of hygroscopic chemicals), if it is assumed that the nonliving material surrounding air-borne bacteria is generally similar in nature. The conditions under which most air-borne organisms are dispersed into the air are such that proteinaceous materials will be associated with them, and these materials generally show a similar equilibrium moisture-relative humidity response. Therefore, it was decided that the more practical expression for the data in Table XXXVI would be in terms of relative humidity rather than in terms of equilibrium moisture content of the air-borne particle.

A study of the data in Table XXXVI shows that there are two definite limiting conditions. As relative humidity decreases, below 50 per cent, the killing power decreases markedly, and, as relative humidity increases above 50 per cent, the killing power rapidly reaches a maximum value, and then levels off. These limitations are explained in terms of the water content of the air-borne particle. At some high value of concentration of solids in the particle, the solubility of the chemical in the particle becomes negligible. Furthermore, the presence of dissolved solids at even the lowest concentrations indicated by the range of relative humidity shown in Table XXXVI is sufficient to prevent the achievement of maximum aqueous solution of the chemical. In either case, solution of the chemical in the air-borne particle is limited, and further condensation of the chemical on the particle will be suppressed.

As previously stated, the effect of relative humidity on the killing power of the chemical as an aerial disinfectant is the major difference between the

action of hygroscopic and nonhygroscopic compounds. In the case of hygroscopic compounds, the humectant concentration of the the compound determines the concentration achieved in the air-borne particle under varying relative humidities. In the case of nonhygroscopic compounds, the equilibrium moisture content of the nonliving material surrounding the air-borne bacteria is the determining factor. Thus, hygroscopic compounds will exhibit their highest killing power at lower relative humidities because a sufficiently high concentration can be achieved to effect lethality; at higher humidities, the aqueous concentration achieved is insufficient to cause the death of the organism. The concentration of nonhygroscopic compounds which can be achieved in the air-borne particle is determined by the water content of the particle; at lower relative humidities, this water content is too low to permit effective solution of the compound and little killing power results, whereas, at higher humidities, a greater concentration of the compound can be achieved through solution in the water of the particle, resulting in greater killing power.

It should be noted that the limitations of solution discussed above apply to both hygroscopic and nonhygroscopic compounds. The difference in expression of the effect of relative humidity on killing power is due to the difference in the factor determining the degree of saturation which can be achieved in the air-borne particle. In either case, it appears to be impossible to achieve the same maximum concentration in the particle that can be achieved in pure water, and, thus, in terms of fractional saturation, it will be impossible to achieve the same maximum killing power in the air as is achieved in vitro. Also, both hygroscopic and nonhygroscopic compounds are limited in their solubility in the air-borne particle as the concentration of solids in that particle increases

beyond a certain point. The difference is in degree--the hygroscopic compound, such as TEG (see appendix), exhibits a high degree of solubility which is reduced only at a very low value of relative humidity; the nonhygroscopic compound, such as 2-ethylhexanediol (1,3), exhibits only partial solubility at an intermediate relative humidity.

6. The Relationship Between Relative Humidity, Aerial Concentration of the Compound, and Killing Power

The equations given in the previous sections were developed for the purpose of relating all the factors which modify the aerial killing power exhibited by a nonhygroscopic compound having a low vapor pressure. Equation 1 expresses the relationship between aqueous fractional saturation of the compound and *in vitro* killing power. Equation 2 relates the effect of fractional aerial saturation of the compound upon the maximum fractional saturation which can be achieved in the air-borne particle at any relative humidity. Equation 3 relates the effect of varying relative humidity upon the maximum fractional saturation of the compound which can be achieved under conditions of aerial saturation of the compound.

Thus, the following equation relates relative humidity and fractional aerial saturation to yield the fractional saturation which can be achieved in the air-borne particle under various conditions of relative humidity and various fractions of aerial saturation:

$$F_P = \frac{(C_{RH})\ (F_{PM})}{100} \qquad (4)$$

where F_P = per cent fractional saturation achieved in the air-borne particle for the particular conditions of relative humidity and fractional aerial saturation of the compound,

C_{RH} = per cent maximum saturation achieved in the air-borne particle under the conditions of the stated fractional aerial saturation (equation 2), and

F_{PM} = maximum per cent saturation achieved in the air-borne particle at the stated relative humidity, the air being saturated with the compound (equation 3).

Having assumed that equal values of fractional saturation in the air-borne particle and in vitro will result in equal values of killing rate, it must follow that

$$F_P = F \tag{5}$$

where F_P = fractional saturation of the compound in the air-borne particle (equations 2, 3, and 4), and

F = fractional saturation in vitro.

This being the case, various conditions of aerial saturation of the compound under various conditions of relative humidity can be equated to yield a calculated k value, the k value having been experimentally determined in vitro.

7. Use of Equations

As stated previously, the equations developed have been prepared to show the relation between aerial disinfectant ability and in vitro toxicity of nonhygroscopic compounds having low vapor pressures. These equations do not describe these relations exactly but they do describe the relationship in such a manner as to be useful in the evaluation of the possible value of compounds which might be considered for use as aerial disinfectants. Although the apparatus for the screening of candidate aerial disinfectants described in this

report is simple and easy to use, it represents special equipment, and, by using it, a considerable amount of time is required to evaluate a compound. The primary purpose of the present section of this report is to propose a method for preliminary evaluation of compounds from *in vitro* data. The collection of such data, although time-consuming, requires the use of routine bacteriological procedures and equipment. In Table XXXVIII, the results of the application of the previous equations to this purpose are presented. The numbers in the body of Table XXXVIII are the various per cent aqueous saturation

TABLE XXXVIII

TABULATION OF EQUIVALENT VALUES OF IN VITRO SATURATION FOR VARIOUS CONDITIONS OF RELATIVE HUMIDITY AND OF AERIAL SATURATION OF NONHYGROSCOPIC COMPOUNDS WITH LOW VAPOR PRESSURES

Relative Humidity Per Cent	Aerial Concentration, in Per Cent Saturation								
	20	30	40	50	60	70	80	90	100
30	47.5	51.5	54.5	57.0	59.0	61.0	62.5	64.0	65.5
40	50.5	54.5	58.0	60.5	63.0	64.5	66.5	68.0	69.5
50	52.5	57.0	60.5	63.0	65.5	67.5	69.5	71.0	72.5
60	54.5	59.5	63.0	65.5	68.5	70.5	72.0	74.0	75.5
70	56.5	61.0	65.0	67.5	70.5	72.5	74.5	76.5	78.0
80	58.0	62.5	66.5	69.5	72.5	75.0	76.5	78.5	80.0
90	59.0	64.0	68.0	71.0	74.0	76.0	78.0	80.0	81.5

values of a compound for which the *in vitro* killing power will be the same as the aerial killing power for the listed conditions of relative humidity and per cent aerial saturation of the compound. These refer only to nonhygroscopic compounds which have a vapor pressure less than that of water. Relations for hygroscopic compounds are presented in the next section of this report.

A brief study of the information in Table XXXVIII shows that certain minimum values of *in vitro* killing power are required of a successful aerial disinfectant. If a compound does not exhibit a high killing rate at approximately 80 per cent aqueous saturation, it will not be expected to be of value as an aerial disinfectant. Within the limits set by the other necessary characteristics of an aerial disinfectant (low vapor pressure, low cost, nontoxic to humans, etc.), such compounds are relatively few in number. The high degree of killing rate required for a *k* value of 0.5 is not usually appreciated; actually this *k* value is equivalent to a kill of 99 per cent in 4 minutes. Such a rate of kill is usually considered as indicative of a very active bactericidal compound. Furthermore, as indicated from the data in Table XXXVIII, a useful aerial disinfectant should also exhibit an appreciable *in vitro* killing rate at not less than 47.5 per cent of its aqueous saturation Again, this is rarely found among compounds having low vapor pressures. The general equation for the relationship between *in vitro* killing power and fractional saturation shows a line having a slope of approximately 6; for the case of a compound exhibiting a *k* value of 0.5 at 80 per cent of aqueous saturation, the *k* value for 47.5 per cent saturation would be expected to be about 0.1.

Although the information set forth in Table XXXVIII is interpreted as indicating that the ideal aerial disinfectant will not be found readily, the present approach should aid greatly in the search for such a compound. In practical use, once the *in vitro* killing power of a compound is determined, the data are plotted on log/log paper, *k* values versus per cent saturation of the compound in water. If the general statement previously made concerning the slope of such a line is true, then only a limited amount of toxicity data will

be required for making this plot, as the slope can be assumed to be approximately 6. Having obtained such a plot of *in vitro* toxicity, the potentialities of the compound as an aerial disinfectant can be estimated by reference to such data as are shown in Table XXXVIII.

B. The Relation Between Aerial Disinfection Power and In Vitro Toxicity for Hygroscopic Compounds

The essential equations for relating aerial killing power and *in vitro* toxicity have been developed in the appendix. As derived, the relationship is primarily determined by the humectant characteristics of the hygroscopic compound. In contrast to nonhygroscopic compounds for which the water concentration in the air-borne particle is the primary factor in determining the concentration of the compound in the particle, the humectant concentration of the hygroscopic compound determines the concentration of this class of compounds in the particle. (For a detailed consideration of these factors, see the appendix.) However, for ease of reference, the essential data are presented here in Table XXXIX. In this table are shown the various per cent of humectant concentration which are achieved in the air-borne particle for various fractions of aerial saturation of the compound. Because these data were developed from equations derived from experiments expressing aerial saturation in terms of maximum saturation of hygroscopic compound at a particular relative humidity, the term "Fractional Aerial Saturation" in Table XXXIX is not expressed in terms of dry air as in the previous section. Instead, it is expressed as the fractional aerial saturation as determined with a glycostat, which determines the modified dew point. Such values are most nearly expressed in terms of a dry air basis by use of the following equation

$$M = (100 - RH) \qquad (6)$$

where M = maximum per cent concentration of the hygroscopic compound which can exist at the prevailing relative humidity, and

RH = per cent relative humidity.

Thus a value of S of 1.00 in Table XXXIX at 80 per cent relative humidity would be equivalent to 20 per cent of the saturation value expressed in terms of dry air, because by definition

$$S = \frac{F_A}{(100 - RH)} \tag{7}$$

where S = ratio of concentration of the hygroscopic compound in the air to the maximum concentration possible at the existing relative humidity, and

F_A = per cent aerial saturation, dry air basis.

TABLE XXXIX

RELATIONSHIP BETWEEN AERIAL CONCENTRATION OF HYGROSCOPIC COMPOUNDS AND CONCENTRATION OF COMPOUND ACHIEVED IN THE AIR-BORNE PARTICLE

Fractional Aerial Saturation (S)†	Per Cent of Humectant Concentrations of Compound Achieved in the Air-borne Particle (P_H)††
0.15	74.0
0.20	76.7
0.40	82.2
0.60	85.7
0.70	86.9
1.00	90.0

† In terms of the maximum concentration possible at the prevailing relative humidity.

†† Determined from equations in the appendix. The values are equal to (0.9) (0.01) C_{RH}, where $\log C_{RH} = \log (100\ S) + 0.9 \log (1/S)$.

Through the use of the values shown in Table XXXIX and a knowledge of the humectant values of the compound at various relative humdities, a direct relationship between aerial disinfection power and *in vitro* disinfection power can be shown. With the exception of relative humidities below 20 per cent, the application of the proper factor from Table XXXIX to the humectant concentration will yield values for the concentration of the hygroscopic compound in the air-borne particle for various conditions of relative humidity and fractional aerial saturation. The actual concentration (C) of the hygroscopic compound in the air-borne particle is determined by multiplying the proper value of per cent of humectant concentration of compound achieved (P_H in Table XXXIX) times the humectant concentration (H_{RH}) of the compound for the existing condition of relative humidity. Thus:

$$(H_{RH})(P_H)(0.01) = C. \tag{8}$$

Assuming equal killing rates for equal concentrations, reference to *in vitro* toxicity data will indicate the expected aerial killing power of the compound under the specified conditions. For example, the approximation equation for *in vitro* killing power of TEG (from the appendix) is:

$$\log (100\ k) = 6.15\ (\log C) - 10.025. \tag{9}$$

Thus, the factors of relative humidity and fractional aerial saturation of a hygroscopic compound are equated in such a manner that a quantitative relationship is shown between *in vitro* and aerial bactericidal power.

VIII. RECOMENDATIONS

The evaluation of aerial disinfectants should be carried out in a simple system, such as the aerosol cylinders described in this report, employing a standard bacterial aerosol. The standard bacterial aerosol should consist of particles of known size, composition, and bacterial content. It is suggested that the standard bacterial aerosol should consist of single-bacterium particles, dispersed from beef-extract broth, using cultures grown in liquid media. It is further recommended that such evaluation be carried out under conditions of temperature between 65° and 80° F and relative humidities of 25, 50, and 75 per cent. In order to maintain a constant standard of reference, it is recommended that the effectiveness of various compounds at 25 per cent relative humidity be compared with that of triethylene glycol; at 75 per cent relative humidity, with that of 2-ethylhexanediol (1,3); and at 50 per cent relative humidity, with both reference compounds.

It is recommended that the biological die-away constant k for specified organisms be employed to express the effectiveness of aerial disinfectants. If proper precautions are taken, S. marcescens should be used as the test organism.

Further work should be carried out in a search for a more nearly ideal aerial disinfectant. In this connection, it is suggested that a combination of compounds might be more effective than any single compound. The potential value of a successful aerial disinfectant is so great that the search should not readily be abandoned.

The available information in the literature concerning the in vitro killing power of various chemicals should be utilized in the search for potential aerial disinfectants.

Under conditions of actual use, hygroscopic compounds should be employed as aerial disinfectants in those situations where relative humidities of 20 to 50 per cent are required, and nonhygroscopic compounds should be employed where relative humidities of 60 per cent or greater are required.

Extreme care should be taken in the interpretation of results obtained from samplers used in aerobiological studies under those conditions where temperatures below 50° F or above 90° F are encountered, and also when extremes of humidity exist.

Further work should be carried out on the comparative evaluation of samplers for aerobiological studies. The significant effects of such variables as relative humidity, temperature both of sampler and of sample, size and composition of the bacterial particle, presence or absence of multibacterium particles, condition of the collected organisms, and composition of the media used in samplers, make it essential that the comparative effectiveness of samplers used in aerobiological studies be determined under a wide variety of conditions. It is further recommended that such comparative studies be carried out in a dynamic chamber such as described in this report.

More extensive studies should be made on the mechanics of sampling bacterial aerosols, especially in an attempt to develop larger volume samplers. It is further recommended that such studies give special attention to the possibility of extending the range of operation of critical-orifice liquid-impinger samplers and thermal-precipitation samplers. The development of a procedure for estimating the absolute efficiency of such samplers would greatly facilitate the progress of such studies.

Further studies should be carried out on the effect of sudden changes in relative humidity on the survival ability of air-borne organisms. Having shown that the sudden change from near saturation to lower relative humidities results in varying survival abilities, a study of other types of changes in relative humidity conditions is warranted.

Further studies should be carried out on the biological factors involved in the survival or death of air-borne organisms. The indication that the "condition" of such organisms is changed in certain situations suggests that the results of these studies might be extremely fruitful in elucidating certain basic factors involved in cellular responses to varying conditions.

IX. APPENDIX

A. Notes on Existing Literature

1. Puck's Equation

In studying the relations between killing power *in vitro* and in air, both at saturation and fractional saturation concentrations, it was decided to formulate concepts without bias rather than to rely on concepts in the existing literature. Having accomplished sufficient experimental work to warrant the formulation of concepts, it was concluded that the killing power of a compound *in vitro* or in air is a function of the fractional saturation of the compound in either case, and that, at equal fractional saturation values, equal killing rates will be exhibited. To check the validity of this concept, other works were reviewed, and, from these, three articles by well-known members in the field were selected for examination. The first of these articles contains work on *in vitro* killing power of TEG, the second contains work on aerial killing power of TEG, and the third consists of a more or less theoretical development of an equation to express the relationships involved in aerial bactericidal effectiveness.

These papers are as follows:

1. "A Study of the Bactericidal Activity in Vitro of Certain Glycols and Closely Related Compounds," by Robertson, O. H., Appel, E. M., Puck, T. T., Lemon, H. M., and Ritter, M. H., J. Infect. Dis. 83, 124-37 (1948),

2. "The Rate of Bactericidal Action of Triethylene Glycol Vapor on Microorgansisms Dispersed into the Air in Small Droplets," by Lester, William, Jr., Robertson, O. H., Puck, Theodore T., and Wise, Henry, Amer. J. Hyg. 50, 175-88 (1949), and

3. "The Mechanism of Aerial Disinfection by Glycols and Other Chemical Agents. II An Analysis of the Factor Governing the Efficiency of Chemical Disinfection in the Air," by Puck, Theodore T., J. Exp. Med. 85, 741-57 (1947).

It must be emphasized that the studies reported in these three papers deal entirely with water-miscible compounds, most of which exhibit a high degree of hygroscopicity, and that the equations developed were intended to explain only the action of such compounds.

The important equation is that of Puck

$$N_g = S\left(1 - \frac{RH}{\gamma_{H_2C}}\right) \qquad (1)$$

where N_g = molecular fraction of germicide in air-borne particle,

S = degree of saturation of the germicide in the air, expressed as the ratio of the concentration in the air to the maximum concentration which can exist at the prevailing RH,

RH = fractional relative humidity, and

γ_{H_2O} = activity coefficient, assumed by Puck as unity.

The data in Lester et al. for TEG in the air are claimed to fit this equation. First, it was decided to determine just how well the equation and the experimental evidence coincide. Since it was not known whether the fractional saturation values in this paper refer to S or to theoretical absolute saturation values, it was necessary to handle the equation for both cases. Treating the fractional saturation values as theoretical absolute yielded values for N_g equivalent to the fractional saturation values, so that regardless of RH, the concentration in the particle would be the same for identical degrees of saturation.

With regard to this fact, plus the observation in the paper that degrees of saturation were determined with a "glycostat," a device that responds to the modified dewpoint, it was concluded that degrees of saturation in this paper were identical with S in the equation. The information obtained by substitution of the reported data in equation 1 is shown in Table XL (Note: For purposes of comparison, if degree of saturation were in theoretical absolute forms, N_g would equal fractional saturation).

TABLE XL

DATA FOR TEG PUBLISHED BY
LESTER, SUBSTITUTED IN PUCK'S EQUATION

Fractional Saturation (Used Here as "S")	Per Cent Relative Humidity							
	25		40		50		60	
	N_g	k	N_g	k	N_g	k	N_g	k
0.15	0.10	0.28	0.09	0.18	0.075	0.075	0.06	0.01
0.20	0.15	0.40	0.12	0.28	0.10	0.13	0.08	0.03
0.40	0.30	0.45	0.24	0.38	0.20	0.25	0.16	0.06
0.60	0.45	0.52	0.36	0.43	0.30	0.28	0.24	0.08
0.70	0.53	0.55	0.42	0.45	0.35	0.29	0.28	0.09
1.00	0.75	0.57	0.60	0.50	0.50	0.30	0.40	0.10

A study of the information presented in Table XL indicates no correlation between N_g and k. If it is agreed that equal concentrations of a compound cause equal killing, then there should be a high degree of correlation, otherwise the equation does not adequately describe the experimental evidence.

In the study of Kaye and Phillips† on ethylene oxide in air, the statement is made "Puck's formula gave an expression whereby the mole fraction of

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† Kaye and Phillips, Am. J. Hyg. 50, 296-306 (1949).

disinfectant was the same for a given concentration of vapor, regardless of the relative humidity." The equation of Kaye and Phillips,

$$W = \frac{p_o}{p} (1 - RH)$$

applies more to Lester's data than does Puck's. However, a greater kill is predicted at increasing RH than is experimentally observed.

The next step was to develop our concepts into an equation which might adequately describe the experimental evidence presented in the papers of Robertson et al. (in vitro studies) and Lester et al. (aerial studies). The object of this effort was twofold: to obtain an approximation equation which would aid in understanding the nature of the interrelationships and to test the concepts of the relationship between aerial and in vitro killing rates with data derived from such an equation.

In formulating an equation, it was assumed that the action of TEG on air-borne organisms is the same as its action on organisms suspended in water. This being the case, then equal concentrations of TEG should cause equal kills, whether the concentration is in an air-borne particle or a water-suspended particle. With this idea in mind, the published data for both conditions from Robertson et al. and Lester et al. were reviewed.

In correlating concentration and k at various RH and partial saturations of compound in the air, it is assumed; (1) that the humectant equilibrium moisture content of a hygroscopic compound will be the limiting concentration of that compound at any particular RH, and that this concentration will be approached, but not necessarily achieved, and (2) that at fractional saturations

less than unity, there will be a complex relationship between the fraction of saturation in air and the resultant fraction of saturation in the aqueous phase of the bacterial particle. Examining the TEG air data from Lester et al. with these thoughts in mind, an approximate relationship is shown in the following equation

$$\log (100S) + 0.9 \log \left(\frac{1}{S}\right) = \log C_{RH} \qquad (2)$$

where C_{RH} = per cent of equilibrium humectant concentration achieved for the particular RH and the particular fraction of saturation in the air, and

S = degree of saturation of the germicide in the air, expressed as the ratio of the concentration in the air to the maximum concentration which can exist at the prevailing RH.

This equilibrium humectant concentration seems to be approximately 90 per cent of the actual humectant concentration at any RH, and so

$$(0.01)(C_{RH})(0.9\ H_{RH}) = C \qquad (3)$$

where C = actual concentration of hygroscopic compound in the aqueous phase of the bacterial aerosol, and

H_{RH} = humectant concentration of the compound at the particular RH.

If the above equations fit the experimental data, for equivalent k values in Table XL, identical values of C should be obtained. Because the equations are approximations made to show the relationship involved, and because of the

inherent variation found in biological data, an exact fit does not result. The underlined data in Table XL serve to illustrate. The three conditions yielding a k value of 0.28 are 15 per cent saturation at 25 per cent RH, 20 per cent saturation at 40 per cent RH, and 60 per cent saturation at 50 per cent RH. Substitution of the proper values from Table XL in equations 2 and 3 yields the following values for C; 69.5, 68.5, and 71.0 per cent, respectively. Plotting the above data on log/log paper and smoothing out the lines for interpolation makes possible checking this in more detail. In every instance, for equal k values approximately identical values of C are obtained. Therefore, it is concluded that equations 2 and 3 describe the relationship between fractional saturation in the air and fractional saturation in the aqueous phase.

From the above relationships, it should be possible to predict the conditions of RH and vapor saturation to yield identical killing rates and to predict, from knowledge of in vitro killing power, the killing rates to be obtained when a hygroscopic compound is vaporized into the air. For the latter condition, it is necessary to know the in vitro toxicity in much the same values as used to express aerial bactericidal power. In the paper of Robertson et al.†, data on in vitro toxicity were presented on an organism which was stated to be similar in its response to TEG as that of the organism used in the aerial work of Lester et al.

Again, an approximate equation was formed to relate concentration of TEG in vitro to killing power

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† Robertson et al. J. Infect. Dis. 83, 124 (1948).

$$\log (100\ k) = 6.15\ (\log C) - 10.025. \qquad (4)$$

The equation has been set so that C in equation 4 is the same factor as in equation 3. This approximation will yield about the same value of k for identical values of C in equations 3 and 4. Thus, the assumption that equal concentrations of a compound cause equal killing rates, whether in water suspended or in air-borne organisms, applies to the data at hand. Such an assumption, of course, does not exclude other possibilities, and the treatment of other data is necessary to determine the exclusiveness of the assumption.

In order to determine the adequateness of equation 4, calculations were performed, treating data for aerial and aqueous killing rates. Substituting these data in equation 2, values for C_{RH} were obtained for various fractions of saturation in the air. These are shown in Table XLI.

Taking equilibrium solution as 0.9 of the humectant concentration (equation 3) values for the actual concentration of TEG in the air-borne particle are obtained. These are shown in Table XLII.

For purpose of comparison, the equilibrium moisture contents of the solids of beef extract (the medium from which organisms were atomized into the air in this work) are shown in Table XLIII.

Now, from equation 4, fitting the *in vitro* data for TEG from Robertson *et al.*, the following points were calculated and plotted for interpolation.

C	k
50	0.0265
60	0.0814
70	0.21
80	0.478
90	0.984

TABLE XLI

RELATIONSHIP BETWEEN PER CENT SATURATION
OF AIR WITH TEG AND CALCULATED† PER CENT EQUILIBRIUM
HUMECTANT CONCENTRATION ACHIEVED IN AIR-BORNE PARTICLES

Per Cent Aerial Concentration	Calculated† Per Cent of Equilibrium Humectant Concentration (C_{RH}) Achieved in Air-Borne Particle
15	82.3
20	85.2
40	91.3
60	95.2
70	96.5
100	100.0

† Calculated by substitution in equation 2:

$$\log C_{RH} = \log(100)S + 0.9 \log\left(\frac{1}{S}\right)$$

where C_{RH} = the per cent of equilibrium humectant concentration achieved for a particular RH, and,

S = the degree of saturation of TEG in the air, expressed as the ratio of the concentration in the air to the maximum concentration which can exist at the particular RH.

At this point, sufficient information is available to warrant a comparison between the rates of kill of TEG *in vitro* and in the air. From the data of Robertson *et al.*, using equation 4, it was possible to interpolate and obtain values for k for various concentrations of TEG *in vitro*. In Table XLI, values are shown for converting fractional saturation in the air into fractional saturation values in the air-borne particle (in terms of humectant concentrations).

TABLE XLII

CALCULATED CONCENTRATION OF TEG IN AIR-BORNE PARTICLES AT VARIOUS RELATIVE HUMIDITIES WHEN THE AIR IS SATURATED WITH TEG

Relative Humidity Per Cent	Equilibrium Humectant† Concentration of TEG	Calculated†† Per Cent Concentration (C) TEG in Particle
20	95.0	85.5
40	89.3	80.4
50	83.0	74.7
60	75.0	67.5
70	61.0	54.9
80	46.0	41.0

† Curme, G. O., ed., Glycols. Rheinhold Publishing Corp., New York, 1952.

†† Calculated from equation 3, for 100 per cent saturation

$$C = (C_{RH})(0.9\ H_{RH})(0.01)$$

where C = the calculated concentration of TEG in solution in the water of the particle,

C_{RH} = the per cent of equilibrium humectant concentration achieved for a particular RH, and

H_{RH} = the equilibrium humectant concentration of TEG at the various relative humidities.

In Table XLII, values are shown for the calculated per cent concentration of TEG in the air-borne particle under conditions of aerial saturation with TEG. Therefore, the information in these two tables is sufficient for the calculation of the expected concentration of TEG in air-borne particles under any condition of RH and of fractional saturation of TEG in the air. These calculations were

TABLE XLIII

EQUILIBRIUM SOLIDS OF BEEF EXTRACT AT VARIOUS RELATIVE HUMIDITIES

Relative Humidity (%)	Equilibrium Solids (%)
20	88.5
30	86.5
40	82.0
50	72.0
60	67.0
70	63.0
80	54.0
90	38.0

carried out and are shown in Table XLIV; the values in the columns headed "TEG Cal. Conc." being calculated from the data in Tables XLI and XLII. In order to relate *in vitro* toxicity with aerial toxicity, the experimentally determined *k* values for *in vitro* kill of TEG (from Robertson *et al.*, interpolated by means of equation 4) are entered in Table XLIV beside the proper value for concentration of TEG, under the heading "*k* Cal." These are the values for *k* experimentally determined for *in vitro* kill but calculated for aerial kill. If the mathematical treatment is correct, then the experimentally determined *k* values for *aerial* studies (Lester *et al.*) should be closely correlated to the *k* value obtained from *in vitro* studies for the same concentration of TEG. The data of Lester *et al.* for aerial kill of TEG are entered in Table XLIV under the columns headed "*k* Exp.," for the proper condition of RH and fractional saturation of TEG in the air.

The limitation of the developed treatment is evident when Table XLIV is examined. Here, the calculated concentration of TEG at various RH and fractions of vapor saturations are shown, along with the equivalent k for this calculated concentration, as well as the experimentally determined k values from Lester et al. (aerial studies) In the range of RH from 25 to 50 per cent, with few exceptions, the calculated k values agree with the experimentally determined k values. (The few exceptions could well be due to calculation errors.) However, at 60 to 70 per cent RH, the calculated k values are larger than the experimentally determined values. This may or may not be significant as all of these values are so small that both calculations and experimental determinations are subject to large variations.

The significant disagreement between the calculated k values and the experimentally determined values shown in Table XLIV is evident at the 5 per cent RH level. Here, the calculated values are approximately twice the experimental values. This fault in the equation is undoubtedly due to a failure of the TEG to act on very dry particles in the same manner as it acts on particles containing more moisture. This failure may be due to noncondensation, nonwetting, or "salting out" of the TEG by the high concentration of solids in or around the bacterial particle. Some support for the nonwetting idea is found in the data of Robertson et al., dealing with the survival of desiccated hemolytic streptococci in redistilled TEG at 10 per cent RH. The k for this in vitro experiment is not greater than 0.05. However, the fact that the data in Table XLIV indicate an increasing k with increasing vapor saturation is not necessarily consistent with this idea. It would suggest that the fall-off of killing power at the low RH is due to the failure of the TEG to dissolve in the high solids concentration in or on the bacterial particle.

TABLE XLIV

THE DATA OF ROBERTSON AND OF LESTER SUBSTITUTED IN OUR EQUATIONS

TEG, Saturation in Air (%)	Per Cent Relative Humidity																	
	5			25			40			50			60			70		
	TEG, Conc. Cal.[1]	k Cal.[2]	k Exp.[3]	TEG, Conc. Cal.[1]	k Cal.[2]	k Exp.[3]	TEG, Conc. Cal.[1]	k Cal.[2]	k Exp.[3]	TEG, Conc. Cal.[1]	k Cal.[2]	k Exp.[3]	TEG, Conc. Cal.[1]	k Cal.[2]	k Exp.[3]	TEG, Conc. Cal.[1]	k Cal.[2]	k Exp.[3]
15	74.0	.30	.07	70.3	.23	.28	66.0	.15	.18	61.5	.09	.08	55.5	.05	.01	45.2	.01	-
20	76.7	.38	.02	72.7	.28	.40	68.5	.19	.28	63.7	.12	.13	57.5	.06	.03	46.7	.02	-
40	82.0	.56	.03	78.0	.42	.45	73.3	.29	.38	68.3	.19	.25	61.5	.09	.06	50.0	.03	-
60	85.7	.74	.35	81.5	.83	.52	76.5	.38	.43	71.2	.24	.28	64.3	.13	.08	52.3	.04	.05
70	87.0	.80	.42	82.5	.58	.55	77.8	.40	.45	72.1	.26	.29	65.2	.14	.09	53.0	.045	.06
100	90.0	.98	.52	85.5	.70	.57	80.4	.51	.50	74.7	.32	.30	67.5	.17	.10	54.9	.05	.07

1. The calculated concentration required to yield the stated "k cal." from the in vitro work of Robertson et al.
2. The equivalent k obtained from the in vitro work of Robertson et al. for the stated "TEG conc. cal."
3. The experimentally determined k value from the aerial disinfection data of Lester et al.

The concept of a limiting solubility in the material surrounding the bacterial particle receives some support from the action of compounds of limited water solubility. The data of Raymond† for iodine as an aerial bactericide were converted into terms consistent with the values used in this discussion and are shown in Table XLV. It can be seen that the killing power of iodine as a vapor increases rapidly with increasing RH above 40 per cent, even at an aerial concentration of about 0.125 per cent of saturation. Iodine has a very limited water solubility (0.029 gm/100 gm water at 20° C) but is an extremely effective germicide at concentrations as low as 0.006 mg/100 gm water. This concentration is about 0.2 per cent of the aqueous saturation value and is of the same order of fractional saturation as that which Raymond showed to be effective as an aerial disinfectant. If it is assumed that iodine is not at all soluble in a solution

TABLE XLV

THE DATA OF RAYMOND FOR AERIAL DISINFECTION BY IODINE†

Per Cent Relative Humidity	k
40	0.02
50	0.07
60	0.18
70	0.45
80	1.00

† 0.1 mg/ft^3, equivalent to 0.125 per cent of aerial saturation.

of solids of the nature of beef extract of the order of 80 per cent solids (such as would be the case at 40 per cent RH), and that the solubility of iodine in

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† Raymond, J. Hyg. 84, 459-61 (1946).

this solution increases as the per cent of other solids decreases, then it would follow that with increasing RH, above some limiting value, the concentration of iodine would increase, with a resulting increase in killing power. (Refer to Table XLIII for equilibrium moisture content of beef extract.) The relation indicated by the plot of Raymond's data is a log/log relation, and the slope of the line is 6.05. Here RH expresses the equilibrium moisture content of the particulate matter, and the possible concentration of iodine in the particle also becomes a function of RH. The slope of the line expressing the relation between killing power and concentration of TEG in vitro has a slope of 6.15 (see equation 4), and reference is made here to the report of Jordon and Jacobs† on the dynamics of disinfection, where, in a similar plot of concentration versus killing rate for phenol acting on E. coli, the line has a slope of 6.60. It does not seem too great an assumption, therefore, to view this plot of RH versus killing rate for iodine vapor as a plot of concentration versus killing power.

The concept of limiting solubility seems a reasonable one because it not only offers an explanation as to why TEG shows a lowered killing power in the air at very low RH, but also it appears to be of value in explaining the increasing killing power with increasing RH of nonhygroscopic, non-water-miscible substances. Thus, limiting water solubility is a factor which must be taken into account before a complete equation can be formulated to describe adequately the variation in killing power with RH. Sufficient data are not available at this time to warrant an attempt at deriving such a complete equation. However, such an equation would include some factor giving significant attention to the effect of increasing

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† Jordon and Jacobs, J. Hyg. 43, 363-69 (1944).

solids content of the air-borne particulate material on the solubility of the aerial germicide. For example, equation 3, which equates the equilibrium effect of fractional vapor saturation upon fraction of humectant concentration actually achieved, illustrates the logical approach. A limiting solubility factor would be included in such an equation in a manner as to indicate a lowered concentration of bactericide (germicide) in the air-borne particle as the concentration of solids increases in that particle. Obviously, some provision would have to be made to include a factor for the free solubility of the particular compound under study.

It can be concluded that there is a very definite relationship between in vitro killing power and aerial disinfectant effectiveness of the glycols. Although at some low RH value the factor of limiting solubility of the glycol in the air-borne particle has not been equated, equations have been presented which adequately describe this relationship. In general, our contention has received support from this study, it being postulated that the killing power of a compound, either in vitro or in the air is a function of the fractional saturation of the compound in either medium. However, originally it had been postulated that, at equal fractions of saturation for either medium, there would be equal rates of kill. This is not necessarily so because RH represents a modifying factor in determining the concentration of the compound in the air-borne particle, and the equations developed indicate that actually equal kills result from equal concentrations in the immediate environment of the bacterium whether in air or in water suspensions. This finding is correlated with studies concerning the nature of the air-borne bacterial particle. These studies indicate that the air-borne bacterium atomized from beef-extract broth (or similar substrates) is

surrounded by a wall of nonliving material making up the actual environment of the bacterium, although air-borne. Thus, it is the concentration of the aerial disinfectant in this immediate environment that determines the fate of the air-borne bacterium. Equal killing rates are thus obtained for equal concentrations of the compound in the immediate environment of the bacterium, whether it be suspended in air or in water.

2. The Action of Propylene and Triethylene Glycols as Aerial Disinfectants

Insofar as the existing literature is concerned, the exact status of these glycols (and, actually, of glycols in general) has been the subject of discussion. Their status has been the subject of one of the few polemics of contemporary scientific literature. The literature is replete with references to the use of these compounds as aerial disinfectants--some propounding their efficacy, others propounding their ineffectiveness. A survey of the literature almost convinces one that it is necessary to subscribe to one of two beliefs: the key to aerial disinfection is either glycols or ultraviolet radiation. In an attempt to evaluate the exact status of the knowledge of the effectiveness of glycols as aerial disinfectants, a critical analysis was made of the most recently published results of laboratory studies. These include the two polemic articles of the Westinghouse group and the Chicago group and two articles from sources not immediately associated with the authors of either of these two groups (Medical Research Council and W. F. Wells). It should be pointed out that only the more recent work of many authors can be considered because various improvements in techniques by these authors have yielded results which indicate that their early work was open to question. No comments are included concerning the open letters

by the two groups[†]. These letters represent the critical opinions of each group for the published work of the other and possibly are not without bias. The following terminology is used in the discussion--"RH" for relative humidity, "PG" and "TEG" for propylene and triethylene glycol.

a. The Westinghouse Group.[††] In the first tests, organisms were atomized into a 4-foot cubical, closed box, the air apparently saturated with PG, but not with continuing vaporization of PG. The data from settling plates are not presented in a manner that permits estimation of apparent particle sizes and counts, and the data from an electrostatic precipitator are shown as a summary and are interpreted to show that no lethal effect exists, but that an increased rate of settling is cause by the PG. Undoubtedly, under these conditions, the supersaturation which was maintained with aerosols of PG in the Chicago group did not exist. The data presented are not expressed in terms of concentration of particles, and it is not possible to make any detailed analysis. However, plotting all the information given in Table I of this paper on semi-log paper indicates that homogeneity was lacking. It is doubtful that there was any significant difference among any of the runs reported in this table, although there might be an indication of a higher k value for some of the runs. However, the fact that the settling plates show no great differences among the various runs can be interpreted as meaning that there was no significant difference. The ratios for the numbers for the first 5-minute count from the precipitator to the colonies settling on plates in 2 minutes were 0.28, 0.3, 0.33, 0.35, 0.28 for the respective conditions: control (50 per cent RH), PG followed by bacteria (65 per

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[†] Science 113, 698-99 (1951)

[††] Nagy, Rudolph, and Mouromseff, Gail, "Effect of Propylene and Triethylene Glycol on Atomized E. coli." Science 112, 593-95 (1950).

cent RH), PG simultaneous with bacteria (75 per cent RH), saturation with PG followed by bacteria (75 per cent RH), and water vapor followed by bacteria (95 per cent RH). Presumably this ratio should be an indication of the $\underline{k}_t$ value; the larger the ratio, the larger the $\underline{k}_t$, whether due to death or to fallout.

In the schoolroom tests, the ratios of the precipitator counts to the settling plate counts (from Table II) for the controls are 2.9, 2.5, 1.8, 1.0, 1.5, 0.6, and for TEG are respectively, 1.1, 1.2, 1.9, 1.2, 1.1, and 1.4. If anything, the comparison of these ratios would indicate a larger particle size for the conditions without the TEG than with the TEG. (There is a possibility that the electrostatic precipitator is very dependent upon gravity settling, and that larger particles are collected more effectively).

The third section of this paper, concerned with the continuous atomizing of a bacterial aerosol in a duct leading into a room, under dynamic conditions, appears more definitive. Sampling was accomplished with Petri dishes positioned throughout the room (20-minute exposure), and a TEG vaporizer was located either in the duct or in the room. No difference in numbers collected is reported, whether TEG vaporized or not. This experiment would seem to show that the commercial vaporizer with TEG was of no value under the conditions of the experiment. It is regrettable that no air samples were taken. Furthermore, the evidence here is open to some question because, in the schoolroom tests, there was an increased number collected on settling plates in the presence of TEG. It could be that the particular vaporizer was ineffective. However, since no air samples were taken, it cannot be proven that the TEG was not taken up by the very large particles which did not "show" under any condition.

Conclusions drawn from Wstinghouse data indicate that the techniques employed leave much to be desired--the raggedness of the data makes it impossible to make a detailed analysis, and the results cast doubts on the value of the electrostatic precipitator for collecting samples of bacterial aerosols. However, this work does seem to demonstrate that commercial vaporizers of glycols as used under the condition of trials have little value for the sterilization of the air and show little, if any, bactericidal effectiveness.

b. The Chicago Group.[†] These studies were carried out in closed chambers, 8 by 10 by 8 feet high. The bacterial aerosol was introduced in a short burst and then mechanically mixed by a fan with the chamber air. PG or TEG was introduced by continual vaporization. Air samples were taken with Folin bubblers, and settling samples on agar plates were exposed for 5 minutes. For each study reported, a control run was made in the absence of glycol vapors.

The data reported were given in sufficient detail to permit further analysis. This analysis was first performed for the control runs. A comparison of the air samples and settling samples by calculating the Petri ratio (number per cubic foot of air, divided by number settling on open Petri dish), and using the nomograph on page 349, Studies in Air Hygiene, (see Section II of this report for this reference) shows an apparent particle size of 7 to 9 microns. Similar values for particle size were calculated by converting numbers settling per minute on Petri dishes to numbers settling per minute per 1,000 cm^2 (Petri dish area multiplied by 15.2) and by converting aerial concentration to numbers per liter; then the rate of fall, in centimeters per minute, was determined by dividing the numbers settling per minute per 1,000 cm^2 by the numbers per liter. In order to

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[†] Lester, Wm., Jr., Dunklin, Edward, and Robertson, O. H., "Bactericidal Effects of Propylene and Triethylene Glycol Vapors on Airborne E. coli." Science 115, 379-82 (1952).

make the resultant particle size obtained by either of these methods of calculations agree with the published particle size of a BMD of 1.5 microns (cascade impactor result), the concentration of organisms in the air would have to be approximately 10 times that obtained in these experiments, or the numbers settling per minute would have to be 0.1 that obtained, or some combination of this. Since it is presumed that the open plates were kept free of contamination, it is more reasonable to assume that the method of obtaining the air sample was only 10 per cent efficient. Even with this assumption, particle sizes estimated from the comparison of the air with the settling samples show a wide range of variation.

Analysis of the runs with glycol vapors indicates a particle size in excess of 50 microns diameter. If, however, the air sampler is assumed to be 10 per cent efficient, a particle size of about 20 microns is determined for the test at 70 per cent relative humidity in the presence of PG; essentially no viables were obtained at 50 per cent RH. The data for the runs in the presence of TEG indicate a particle size range of 10 to 20 microns if the air samplers are considered to be 100 per cent efficient.

If the counts given are assumed to be correct, the die-away for the control run is almost entirely due to fall-out, the k_f † being about 0.2. If the bubblers are assumed to be 10 per cent efficient, k_f is 0.0016. For PG at 70 per cent RH, if counts given are correct, k_f is 0.8--a figure greater than the total. If the bubblers are 10 per cent efficient, k_f is 0.093. It is our conclusion that this type of bubbler is about 50 per cent efficient. If this is the case, at 70 per

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† See section C for equations used.

cent RH, for the control, k_f is 0.0045 for a particle of 3.8 microns in diameter, leaving a net k due to death alone of 0.021. Under the same conditions for PG, k_f is 0.20 for a particle size of 27 microns. Subtracting the control k due to death alone and the k_f, the net k due to death caused by the PG alone is 0.06.

The PG run at 50 per cent RH is at a higher concentration of PG. Here the combination of fall-out and lethal effect combined to yield essentially sterile samples. However, the fact that any viable organisms were collected on the settling plates during the first 6 minutes (when no organisms were demonstrated in the air samples) indicates that the particles were quite large.

Conclusions drawn from the Chicago work indicate that PG has a definite bactericidal effect, but most of its effectiveness in cleaning the air of air-borne bacteria is due to the settling out of these particles, and this requires the presence of an aerosol of the PG in order to overcome the constant loss of PG by condensation on walls, etc. The conditions under which the Chicago work was done were the most conducive to demonstrating large apparent k values. The organisms were introduced into an atmosphere having a high concentration of PG aerosol, and immediate precipitation occurred. It would seem as reasonable to assume that differences in the physical condition of the bacterial particle with varying RH are responsible for the dependence of k upon RH as to assume that a differing lethality is involved.

c. Medical Research Council (London).[†] The studies on the effect of chemical vapors on air-borne organisms were carried out in a closed room 10 by 8 by 10 feet high. A 10-inch fan was used to mix the short burst of bacterial

[†] Bourdillon, R. B., et al., Studies in Air Hygiene. Medical Research Council Special Report No. 262, H. M. Stationery Office, London, England, 1949.

aerosol with the room air, as well as to mix the chemical vapors. Samples of the air were collected with a slit sampler, and settling samples were taken with open Petri dishes. The general procedure was to adjust the room to the proper temperature and humidity, then, with the fan running, to disperse the bacterial aerosol. The control information was taken thereafter for 2 minutes, followed by vaporization of the compound under study. Further sampling was carried out for 4 minutes after the compound was vaporized.

The results of their studies expressed as K, which is in hours, and is 138 times the biological k; the K for death is given as the net value after deduction of the control K. This K is determined by aerial samples plotted on semi-log paper and is 138 times the slope of the line for the first 90 seconds after the line shows a break due to initiation of kill. In this book, a section written by Lidwell, Lovelock, and Raymond is devoted to a study of PG. They used concentrations of PG which appear to be below saturation, and found a direct correlation between rate of vaporization of the glycol and the resultant K values, there being no correlation between concentration and K values. The maximum concentration achieved was 10 mg/ft^3 (compare this with the Chicago work at such supersaturation values as 14.7 mg/ft^3). In those experiments where they evaporated PG in large quantities and at great rates, they did obtain very large K values (250 and over), even though the reported concentration in most of these instances was below the maximum. (The condensation of PG on the walls of the chamber is on p. 125 and shows that the concentration drops readily from saturation values to some lower value, which dies away more slowly.)

A study of settling rates is presented for several RH values at a PG concentration of 1.0 mg/ft^3. At 38, 57, and 87 per cent RH, they obtained K values of

38, 90, 70, and 31, and K_s of 6, 9, 62, and 27, respectively. (It was suggested in the text that high K_s at 71 per cent was in error.) These data indicate a higher net K_d at 57 per cent than at other RH, but their explanation does not consider this. From the results of in vitro experiments and calculations of the concentrations of PG in the bacterial particles, they conclude that below 60 per cent concentration of PG the kill is very slight, and that at RH above 60 per cent the bacterial particle does not contain this much PG, therefore, considerable condensation can take place at higher RH without causing death, although causing an increase in particle size and fall-out. They conclude ". . . the bactericidal effects of vaporizing propylene glycol are complex, and that under certain circumstances the killing rate attained is apparently dependent on phenomena of relatively short persistence, possibly associated with supersaturated conditions in the vapor. Insufficient data are available to present a complete picture of its mode of action, and the small probability of any extensive use being made of propylene glycol as an aerial bactericide, owing to the excessive quantities required, makes further work to this end of doubtful value."

Conclusions drawn from the Medical Research Council work indicate that their work is of excellent quality and well analyzed. In general, their data are more nearly in agreement with those of the Chicago group; however, their analysis is much more thorough, and a clear distinction is made between lethal effect and increases in settling rates.

d. Wells, W. F.[†] The reported studies were carried out in a series of dynamic chambers, each having a detention time of 1 minute. Death rates were

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[†] Wells, W. F., Airborne Contagion and Air Hygiene. Harvard University Press, Cambridge, Mass., 1955.

determined from data obtained from air samples taken with the Wells centrifuge, from inlet and outlet of each chamber. These data are reported as lethes. A lethe is defined as the equivalent of the effect of ventilating one volume change in 1 minute, or the effect which will remove 63.2 per cent of the air-borne contaminants (including bacteria). A lethe for 1 minute is 2.3 times the biological k, being expressed as the natural logarithm, rather than logarithm to base 10. However, since the lethe is defined in terms of 1 minute, there is no direct comparison between the values for biological k and lethe for any other period of time. Thus, a total of lethes for 2 minutes (as used by Wells) is the negative Naperian logarithm of the per cent of survivors, and, to convert to biological k values, it is necessary to divide by (2) (2.3). The information for PG and TEG reported by Wells in "Appendix Two" of his book can be converted by these means to a common method of expression for comparison.

In general, the data presented by Wells indicate a very high rate of disappearance of organism when either PG or TEG is introduced into the air, this rate diminishing as the RH increases from 40 to about 80 per cent. However, the reported values were determined from air samples; therefore, there is no method for estimating what portions of these rates were due to fall-out and lethal effect. Furthermore, the compound under study was introduced at the inlet to the first chamber, and it is probable that the concentration of compound in the second chamber was lower than the initial value, due to condensation losses. This factor probably accounts for the reported lower value for lethes for the second chamber ("second minute"). However, on the basis of a continued fractional disappearance (as postulated by a logarithmic die-away), Wells' data for the "second minute" are not as greatly different from those for the "first minute" as are

indicated by the values for lethes. For example, values reported in lethes for the "first minute" and "second minute" of 1.37 and 0.87, respectively, (25.0 and 10.0 per cent survivors) are calculated to yield k values of 0.6 and 0.5. It may well be that this type of apparent difference is responsible for statements of Wells concerning a very high initial death rate caused by glycols.

It is concluded from the work of Wells that, because of the method of presenting information and because fall-out is not considered in the calculation of the presented data, the values reported by him for rates of disappearance of bacteria from the air in the presence of PG or TEG are not directly applicable to a discussion of the causes of this disappearance. However, the numerical size of the reported values indicates a degree of effectiveness comparable to that reported by the Chicago group.

e. Conclusions Drawn from an Analysis of the Literature on the Action of the Glycols as Aerial Disinfectants. The following conclusions are drawn:

(1) PG and TEG when present in the air at concentrations approaching saturation have a decided effectiveness in cleaning the air of bacteria. This effectiveness is most pronounced at relative humidity values between 30 to 70 per cent.

(2) A large part of the effectiveness of PG and TEG in cleaning the air of bacteria is due to condensation of the glycol on the air-borne particle and the subsequent deposition of the enlarged particle.

(3) Particles of air-borne bacteria, enlarged and settled as a result of glycol condensation, probably contain viable organisms if this phenomenon occurs at relative humidity values of 60 per cent and greater. However, it would be expected that if the relative humidity is subsequently reduced, such organisms would be killed by the increased concentration of glycol.

(4) Although the above conclusions appear reasonable in the light of the results from several different studies, from several laboratories, the methods and experimental planning of the various workers leave much to be desired in view of conclusive information as to the exact action of glycols as aerial disinfectants.

B. Physical Factors Involved in the Operation of Liquid-Impinger Samplers

The so-called critical-orifice, liquid impinger has proven to be the most flexible sampler for experimental aerobiology. However, attempts to extend the range of operation of this type of sampler have not been entirely successful. There are definite indications that the effectiveness of sampling is lowered as the volume flow is increased. Data obtained early in the course of this work showed that efficiency decreased whenever critical orifices having a rate of flow in excess of 1 liter per minute were used. Illustrative data are given in Table XLVI. Although the comparative values show considerable scatter for the various ranges of impinger capacities, there is a general trend towards lower and lower recoveries as the flow rate is increased. It should be noted that these data were obtained when using plain-gelatin solution in the impinger bottles; more recent data obtained with enriched fluid do not show such a marked difference between the 1-liter-per-minute and the 5-liter-per-minute samplers.

The data presented in Table XLVI were used initially as the basis for standardizing operations with a 1-liter-per-minute sampler. However, interest in extending the range of operation of the critical-orifice, liquid impinger continued throughout the term of the project, and insofar as time permitted, certain studies were made on the mechanics of operation of these samplers. A consideration of the situation existing in the operation of these samplers

TABLE XLVI

RESULTS FROM THE USE OF VARIOUS-SIZED, CRITICAL-ORIFICE, LIQUID-IMPINGER SAMPLERS UNDER IDENTICAL CONDITIONS†

Sampler (l./min)	Number of Viable Organisms per Liter of Air
0.90	4800
0.95	6500
1.00	4800
1.15	5000
1.20	6000
1.25	5000
1.30	5000
1.35	5500
2.00	5500
2.40	4800
2.50	4000
2.60	4800
5.00	3500
5.10	3000
5.15	4200
5.20	3000
5.25	3600
5.30	4200
5.35	4200
41.0	1800
42.0	2400
42.5	1300
43.0	2200
44.0	2400

† Dry bulb 76° F, RH 58 per cent.
Impinger medium: Plain gelatin, buffered.

indicated that impaction alone could not explain their ability to collect small paricles from the air mainly because the collecting liquid is forced away from the tip of the impinger and does not present an unyielding surface against which efficient impaction can occur. High-speed photographs taken of the action occurring during sampling showed a similarity between this action and that involved in bubble formation. A thorough study of this mechanism was made, and it was concluded that the efficiency of the critical-orifice, liquid impingers must be due to a combination of impaction and scrubbing action. The following section gives, in some detail, the analysis of this action; high-speed photographs are then presented to illustrate this analysis. In addition, certain physical characteristics of sampling fluids were determined in view of the proposed mechanism of sampling. These are presented at the end of this section.

These studies were not pursued further. However, the studies and the data presented will be of value in future attempts to extend the useful range of operation of critical-orifice, liquid impingers.

1. Theoretical Approach

The recovery of fine particles from an air stream being projected into a liquid medium may be essentially a mechanism of inertial settling. If this is true, there are probably two stages in which this inertial settling takes place, with each stage being affected by the rate of flow of the air containing the aerosol and the physical characteristics of the liquid into which this flow is projected. The first stage would be primarily one of early velocity differential where the forward inertia of the particles would be slightly greater than that of the carrying air (so slight that possibly the particle would only have to be as much as one radius removed from its initial carrier) such that the particles

or clump of particles would be subjected to an immediate liquid surface and deposited thereon, depending on the attraction of the liquid surface for the particle. This phenomenon is referred to as impaction in the remainder of this discussion. In the second phase, a much slower and involved activity is encountered, and inertial settling is dependent on interchange at liquid-aerosol interfaces formed by bubble generation and scrubbing action. Recovery of particles in this phase is then dependent upon the factors governing entrapment and solubility of air-particles in the liquid and is enhanced by the maximum occurrence of aerosol-water interfaces and interchange at these interfaces.

The subject is divided into the two stages, with each stage being further subdivided into activities primarily concerning the jet of particle-carrying air and those of the liquid. No attempt is made to separate sharply the stages or the activities within each stage, for, in order to establish a complete picture, each must be considered in their interrelationships.

In the following discussion, no references are made to the literature concerning direct impaction. Extensive reference is made to the literature concerning the mechanics of bubble formation, especially to a review by Paul D. Haney† and to the original articles reviewed therein.

a. Impaction-Velocity Differential. One of the first and primary requirements in operating a collector of the orifice liquid-impinger type is the necessity for accurate metering of flow per unit of time so that reproducibility may be achieved. Obviously, it is desirable for the collector to be operable without making continuous flow measurements, thereby eliminating hazards of leaks,

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† Haney, Paul D., J. Am. W. W. Assn. 46, 365 (1954).

etc., in conjunction with the measuring device. When air is drawn or projected through a tube having a capillary- or orifice-type restriction at the exit so that sonic velocity is attained by the issuing air, it is generally recognized that the orifice operates as a critical flow orifice. As long as the driving force required to produce flow at sonic velocity is maintained, the rate of flow through the orifice may be accepted as constant. Repeated calibration of liquid impingers operated as critical orifices have shown their air flow per unit of time to be highly reproducible--at least within the limits of measurement with the wet-test meter.

The velocity at which air and particles emerge from the orifice primarily determines the extent and strength of movement in the downward or forward direction and, therefore, the force with which they contact any interrupting surface. It is reasonable to assume that the greater the speed with which the air and particles contact the surface of the liquid the greater will be the resultant impaction. From a practical standpoint, sonic velocity is the highest velocity that can be consistently imparted to the air stream.

In conjunction with rate of flow through the impinger orifice, the configuration and size of the jet may affect the action occurring at the contact surface of the jet stream and liquid. There is some reason to believe that there may be advantages in using smaller diameter orifices. If it is assumed that the concentration of particles per unit volume of air is constant, a small jet stream may increase the probability of immediate contact occurring for a given surface contact area. Perhaps there may be less drag on the outer areas of the small jet stream than may be true of the larger jet stream.

Consideration may also be given to the location of the sampling-tube tip in relation to the vertical and lateral dimension of the liquid container. This is probably related not only to the volume of the liquid but also to the volume rate of sampling. In this part of the discussion, primary interest is in impaction on the liquid surface, and, hence, the physical relation of the tip to the container and liquid volume should be such that the issuing jet is always presented with a liquid surface. With high-velocity jets, there is considerable turbulence, oscillation, and sometimes spiraling which may result in some "throw out" to the sides. A certain minimum volume of liquid is required, in relation to the volume rate of sampling--otherwise, the entire liquid can be suspended by the air stream. The distance from the sampling tip to solid surfaces of the container must not be less than some definite value, depending on the volume rate of sampling and the velocity of the jet. It the tip is too close, it is presumed that some of the particles stick to the container surface, or that re-atomization may occur due to the impaction force. These factors suggest that a minimum volume of liquid, a definite size and shape of the container, and a directed location of the sampling tip are essential to assure maximum exposure of the jet stream to the liquid surface.

The action of the orifice-type, liquid sampler as an impinger is also dependent upon the constitution, inherent properties, and surrounding conditions of the liquid itself. From the standpoint of rigidity and adhesiveness of the collecting surface, the liquid should be of such composition as to resist deformation by the air jet and yet at the same time should possess characteristics which enable the liquid to wet and entrap the impinging particle.

If maximum impaction or friction is desired when the aerosol contacts the liquid surface, the degree of collection may well be dependent upon the viscosity of the liquid. The cohesive forces which work towards keeping the molecules of the liquid close together will largely determine the rigidity and resistivity of the liquid surface. In order for the impinging particle to be retained by the collecting surface, it is essential that the forces governing attraction of the liquid and particles for each other be such that the particle be wetted and entrapped upon contact with the liquid surface. Particles are wetted by the liquid when the attraction of the particle molecules for those of the liquid are greater than that between the molecules of the liquid for themselves. That is, the adhesive forces tending to bring together the molecules of the liquid and those of the particles exceed the cohesive forces maintaining union of the molecules of the liquid. This suggests a liquid with a comparatively high viscosity and low surface tension.

The temperature of the collecting liquid medium may be of concern from the standpoint of effect on surface tension and viscosity. In general, the viscosity of a liquid decreases fairly rapidly with increasing temperature, decrease per degree rise being greater in the temperature range of 35° to 100° F than in the higher ranges. Surface tension also decreases with increasing temperature, the decrease being greater in the higher temperature ranges.

Changes in the collecting-fluid viscosity may also be caused by the high shearing action developed in liquid impingers. For example, the viscosity of gelatin gels is markedly reduced by such shearing action.

In addition to the normal impaction of particles on the liquid surfaces, there may be slight collection based on chance collision of particles in the air stream with fine particulate matter in the liquid.

b. Bubble Generation, Scrubbing, and Interchange at Interfaces. If we assume that there is recovery of particles over and above that portion collected by direct impingement, this additional recovery must depend upon the action of the sampler as a bubbler and/or scrubbing device. It may be well to consider the action of the sampler by which air-borne particles can be collected through bubble formation and the factors affecting the efficiency of collection by this method. The theory of bubble formation may be a complex one, but it is interesting to consider its aspects in general terms. This consideration includes conditions conducive to bubble formation, factors regulating bubble size, and the various phenomena by which a part or all of the contents of a bubble may transfer to the liquid in which the bubble is formed.

When a stream of air or gas (air, gas, and aerosol are terms used rather interchangeably throughout this discussion) is projected into a liquid medium, the surface of the liquid resists the addition of this gas. The resistance offered by the liquid is dependent to a large degree upon the solubility of the gas in the liquid. If the gas is not instantaneously soluble in the liquid, then the volume and force of the gas builds up against the liquid surface, and the surface is distorted until the volume of gas is virtually surrounded by a liquid skin A point is then reached where the partially surrounding film of liquid tends to rejoin itself so strongly that the volume of gas is finally encased in a spherical skin of liquid. Then buoyant forces acting on the bubble formed reverse the downward motion and enhance any upward motion of the bubble, causing it to rise toward the surface of the liquid. The transfer of constituents of the gaseous interior of the bubble is then dependent upon the factors controlling interchange of constituents at the gas-liquid interfaces,

and, after the bubble has risen to the surface, upon the conditions of the vapor existing above the surface of the liquid.

The particles not separated earlier from the carrying air by differential velocity will be contained in the bubbles formed, and the recovery of particles at this stage is dependent upon the factors stated previously. Again, the mode of collection is essentially that of inertial settling It differs from that occurring with impaction in the source of the force imparting inertia to the particle and from the manner in which surface contact is made.

Undoubtedly, the transfer of a part or all of the constituents of a bubble to the containing liquid takes place at the point of the most direct contact between the two surfaces, i. e., at the gas-liquid interface. The degree of transfer depends upon the thickness of the film formed at the interface and the forces prevalent to maintain the film. Transfer is enhanced by conditions which increase the interfacial area and reduce or break down the separating boundary.

Assuming that the activity of collection is at the gas-liquid interfaces and that the maximum activity occurs when the interfacial area is greatest, it may be seen that the size and number of bubbles per volume of gas is related to transfer of constituents of the bubbles into the containing liquid. Many factors may influence the size and the rate of bubble formation. The rate of gas flow through the orifice may be considered. High velocity creates a continuous chain bubble formation, and also it provides agitation which decreases resistance to gas transfer. Bubble size is dependent to a large extent upon the diameter of the orifice, the smaller orifices producing smaller bubbles. The smaller bubbles have a comparatively large surface area in relation to volume; thereby, they form large interfacial surfaces when in contact with the liquid. Haney states that

the thinnest films are apparently encountered immediately after the formation of a bubble and that the rate of diffusion is a maximum at the moment of formation of the gas-liquid surface. Therefore, it is important to create new gas-liquid interfaces, and this may be achieved through high rate formation of small bubbles.

The size of the bubble formed may also indirectly affect the thickness of the interfacial film; the buoyant effect is greater on a small bubble than on a larger one, resulting in an increase in the terminal velocity of the small bubble, which tends to maintain the film at its thinnest condition There may be contradictions to this under certain circumstances, but it probably holds true for those cases where the surface area per unit volume is the controlling factor as the ratio of surface area to volume of a sphere increases in an inverse relation to diameter. Also, the benefit derived from a rapidly moving bubble may be offset somewhat by other factors, such as the shortness of time the bubble is exposed to the liquid surface.

The relation of the volume of air per unit of time and total volume drawn through the sampler to the volume of liquid in the sampler influences the solution of gas and the recovery of particles. If the volume of liquid is so small that the incoming gas cannot be contained within the liquid for even a short interval of time, there may be direct escape of the gas without its having been in contact with the liquid surface. Violent agitation under these conditions results in open spaces in the liquid where the gas is drawn out ot the sampler by the vacuum source without opportunity for impaction or solution. Under certain conditions, the entire liquid might be suspended by the gas stream. Then, too, with the small liquid volume, the amount of new liquid surface exposed to

the bubble formed and the length of the path the bubble travels in the liquid is limited. This reduction in free area and free path in which the bubble travels may result in bubble coalescence. Also, with this large gas to liquid ratio, the saturation limits of the liquid for the gas may be reached. The equilibrium concentration or saturation value of a gas in comparison to its actual concentration is one of the driving forces available to cause interchange between the gas and the liquid.

Maximum chance for interchange between two surfaces may occur when there is considerable drag or friction between them, with force sufficient to impart a favorable motion of one with respect to the other. Friction of a moving body is defined by Stokes' Law of viscosity "resistance is proportional to the surface area of the body and the velocity at which it moves through the medium." This may suggest the desirability of the coexistance of a viscous medium and small bubbles. The small bubbles represent a relatively large surface area and are strongly pushed along by buoyant forces. In such a case, maximum drag or friction combined with maximum or optimum surface contact area enhance the chance for interchange at the interfaces. Another factor worthy of note at this point is the surface tension of the liquid medium which influences the wetting ability of the liquid. A liquid combining high viscosity and low surface tension may be ideal.

The internal temperature of the sampler during the operating period may affect the efficiency of collection. Factors such as viscosity, surface tension, and general gas solubility are somewhat dependent upon temperature. As stated earlier, viscosity decreases with increasing temperature, and, at higher temperatures, liquids give up entrapped gases rather rapidly--especially where a partial

vacuum exists. Also, the surface tension decreases with rise in temperature. In addition, the pressure and the humidity present in the sampler are somewhat dependent upon temperature. All of these factors are generally recognized as affecting the solubility of a gas in a liquid and transfer of particles from the gas to the liquid.

The pressure and humidity present in the sampler may affect the recovery efficiency of the sampler. Critical-orifice, liquid impingers are usually operated by vacuum, and, therefore, there is a negative pressure above the surface of the liquid which minimizes the possibility of collection after the bubbles and particles have traveled up through the liquid. For pressures up to one atmosphere and for gases which do not react chemically with the liquid to any extent, the amount of gas which will dissolve or diffuse into the liquid at a given temperature is directly proportional to the partial pressure of the gas in contact with the liquid. Notwithstanding other created conditions which may be unfavorable to the survival of organism, this suggests that operation of the sampler under conditions of positive pressures may be more favorable to efficient collection. Under positive pressure, the activity above the surface of the liquid would play a more important part in collection. Even under negative pressure, there may be limited recovery of particles at this phase of activity. The gas is compressed prior to issuance from the orifice. As it expands on leaving, considerable cooling takes place, creating a condition favorable to condensation of vapor in the space above the liquid surface. This vapor caused by the carry-out of liquid from the released bubbles may actually result in some deposition of moisture on the particles themselves. (Raoult's law defines condensations as the rate of molecular exchange between a vapor at the surface of the liquid.)

If there is much condensation of the vapor and wetting of the particles, then it may be possible for some of the particles to settle (size of the particles would be increased by addition of moisture) or to be collected by subsequent splashing liquid thrown into contact with them by the violent agitation of the liquid occurring during sampling. An atomized droplet which has evaporated somewhat when dispersed in the air, making up an aerosol cloud, may regain moisture quite readily under favorable conditions. From the standpoint of building up the size of the particles, humidification prior to sampling may be worthy of consideration. One may imagine collection with critical-orifice, liquid impingers as "reverse atomization." Thus,

Atomizer: liquid → atomization → droplet → evaporation → droplet nucleus.

Impinger: droplet nucleus → collection → wetting → condensation → liquid.

Although the presence of fine solids suspended in the liquid may be of greater importance in connection with impaction, there is the possibility of additional action resulting from produced changes in the surface tension of the liquid and increased drag with lowering of the terminal velocity of bubbles, especially small bubbles. It is also indicated that surface-active agents might be of value in increasing the efficiency of collection.

In this discussion, the terms air, gas, and aerosol have been used rather loosely and interchangeably. However, it should be emphasized that an aerosol may or may not behave as a gas, depending upon its composition and the conditions under which it prevails.

The following are the important variables involved in the collection of air-borne particles by liquid impingers:

1. Clumping (break up of clumps).
2. Paticle size.
3. Configuration of orifice.
4. Size of orifice.
5. Velocity at orifice.
6. Volume flow rate of sampler.
7. Distance from tip of orifice to solid surface.
8. Vertical location of orifice in relation to liquid.
9. Vertical and lateral dimensions of container (shape of container).
10. Volume of liquid in sampler.
11. Viscosity of liquid.
12. Surface tension of liquid.
13. Addition agents such as antifoam, etc.
14. Density of liquid.
15. Particulate matter in liquid.
16. Internal temperature of sampler.
17. Pressure in sampler.
18. Humidity (both prior to sampling and in sampler itself).
19. Liquid loss during sampling.
20. Sampling time.

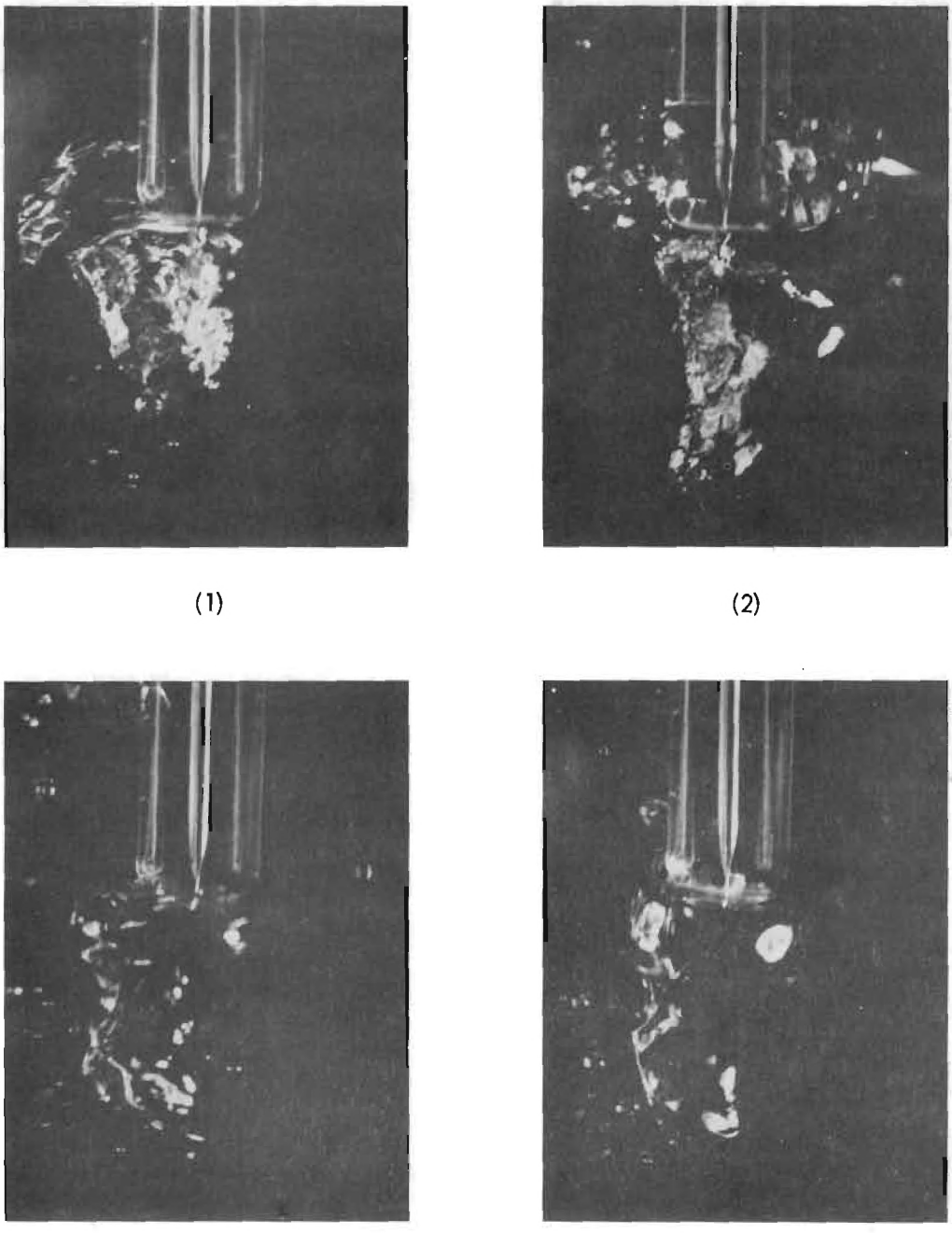
In addition to the above list, biological factors must also be considered.

2. Analysis of High-Speed Photographs Taken of the Action of Critical-Orifice, Liquid Impingers

In order to distinguish the details of the formation of bubbles during the operation of a critical-orifice, liquid impinger, it was necessary to make studies on a low rate of flow orifice, otherwise the violent agitation taking place in the liquid completely obscured the details of action. For the purposes of photography, a 0.293-liter-per-minute orifice was employed. This orifice was formed by constricting the end of a 1.47-mm-diameter capillary to approximately an 0.18-mm diameter. The ratio of the capillary (which was the throat of the stream prior to the orifice) to the orifice was thus approximately 8:1, being well above that required for critical operation. In operation the upstream pressure was measured as 74.0 cm of mercury; the downstream measured as 26.6 cm of mercury. This yields a pressure ratio of 0.36, which is well below the value for critical operation for air at room temperatures.

The first photographs were taken on 16-mm film at 4,000 frames per second. Three reels were exposed and subsequently studied. A tentative series of events was determined from these films. Subsequently, over 100 exposures were made with a Leica camera and a Strobelume light source at 4-ms exposure. Because these photographs were suitable for reproduction, representative pictures were selected to show the serial action as determined from the study of the motion picture film and are shown in Figure 18.

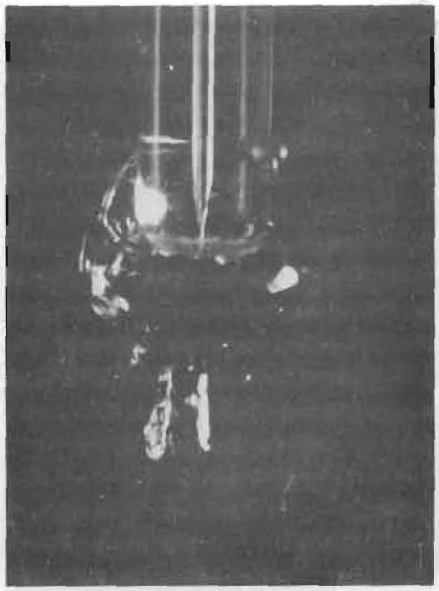
In the photographs Nos. 1 through 12, the suggested series of events is as follows: the jet issuing from the orifice is unstable, oscillating about 15 times per second, so that sometimes a bubble is formed directly at the tip of the jet, and other times the jet simply breaks up into numerous smaller bubbles. When a large primary bubble is formed, it grows to a definite size, then is



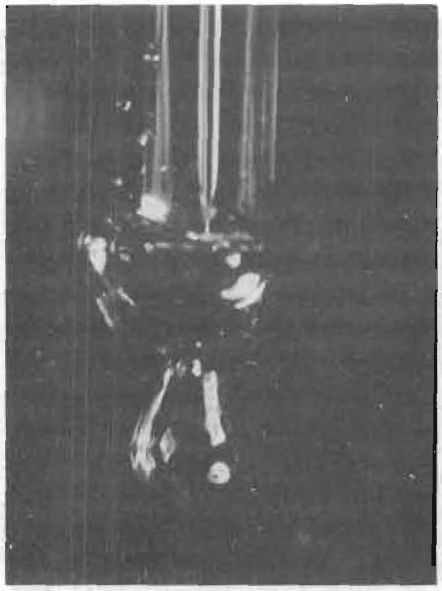
(1) (2)

(3) (4)

Figure 18. High-Speed Photographs of Action of Critical-Orifice, Liquid Impinger.



(5)

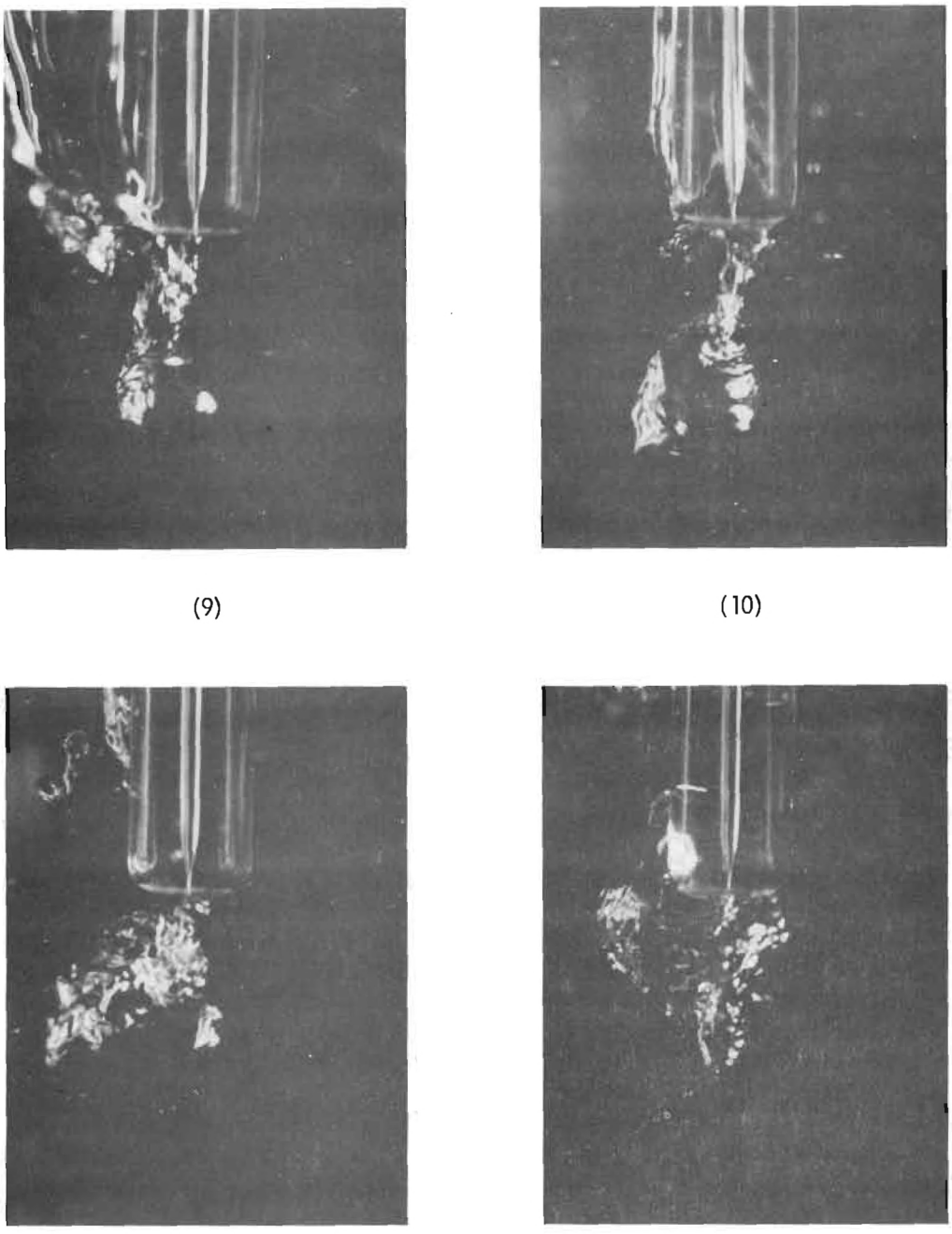


(6)

(7)

(8)

Figure 18. High-Speed Photographs of Action of Critical-Orifice, Liquid Impinger.



(9) (10)

(11) (12)

Figure 18. High-Speed Photographs of Action of Critical-Orifice, Liquid Impinger.

buoyed to the surface, and the process is repeated. The rate of formation of the large bubbles is about 10 per second, and about 200 small bubbles are formed each second. It is the combination of these effects that seems to account for the ability of the critical-orifice, liquid impinger to remove particles from the air stream. There must be some inertial separation as the jet issues from the tip and a great deal of scrubbing action as the smaller bubbles break off and are projected through the liquid. In general, the sequence of events suggested in these photographs embodies the actions discussed in the first part of this section.

There is one point of difference between the events depicted in these photographs and those seen in the photographs of various authors dealing with bubble formation. This is the formation of a definite jet or stem at the exit of the orifice, definitely discernible in the present photographs. The reason for the occurrrence of this jet under the conditions of critical operation is due to the greater velocity of the air stream in the present instance as compared to that used by other workers. At rates of flow through an orifice of much less than acoustic velocity, the buoyant effect will be much greater than the impaction effect, and bubbles will form at the orifice face and immediately rise to the surface. However, at acoustic velocity, the force of impaction will be several times greater than the buoyant effect, and it would be expected that an elongated bubble (or jet) would be formed initially. This is exactly the case as can be seen in several of the photographs in Figure 18.

3. Certain Physical Characteristics of Sampler Fluids

During the course of these studies numerous observations and measurements have been made on the physical characteristics of sampler fluids. Although

time has not permitted the successful correlation of these data to any useful conclusion, they are included at this point as they might be of some value in later investigations of the mechanism of the operation of critical-orifice, liquid impingers.

The surface tension values for several fluids are given in Table XLVII. These values were obtained with a Cenco du Nouy Tensiometer (direct reading, with a 4.0-cm loop). All readings were made after rinsing and cleaning the loop until the distilled water reading was between 70.5 and 71.0 dynes. It appears that the silicone in the AF mixture interferes with the estimation of surface tension; after filtration (presumably removing the insoluble silicone), the effect of the emulsifying agent in depressing the surface tension is evident for all fluids except the plain gelatin. It is possible that this is a clue to the enhanced ability of the more complex fluids in collecting air-borne bacteria, in addition to any biological effect.

The results of viscosity measurements made on several fluids are shown in Table XLVIII. These measurements are the average of five measurements each with two different Oswald-Fenske pipettes. It is possible that the slightly higher viscosity values for the more complex fluid may be a further factor in explaining the greater effectiveness of this fluid in collecting air-borne bacteria.

C. Mathematical Expressions Employed

The expression k is used to denote the rate of loss of viable organisms, from all causes. This expression is employed by biological workers to express

TABLE XLVII

MEASUREMENT OF SURFACE TENSION OF VARIOUS IMPINGER MEDIA[a]

Media	Temp. (°F)	Dynes per Centimeter		
		Untreated	Addition of DOW AF[b]	Addition of DOW AF and Filtered
Distilled Water	78	70.5	----	----
Peptone-yeast-gelatin[c]	78	45.1	33.0	34.1
Gelatin (0.2%)[d]	78	57.3	36.0	44.3
Beef extract[e]	78	49.0	----	30.3
Brain-heart-gelatin[f]	78	49.0	----	31.6

a. Measurements made with the Cenco du Nouy Tensiometer, direct reading, 4.0-cm loop.

b. DOW AF - DOW Corning Antifoam AF Emulsion - a water dilutable dispersion of DOW Corning Antifoam A silicone defoamer. (DOW Corning Corp., Midland, Mich.)

c. Peptone-yeast-gelatin - Pharmagel A, 2 grams (buffered), proteose peptone, 20 grams, yeast extract, 3 grams, sodium chloride, 2.5 grams, distilled water q.s. to 1000 ml.

d. Gelatin - 0.2 per cent Pharmagel A solution buffered with 0.08 gram Na_2HPO_4 per gram of gelatin, adjusted with 10 per cent KOH to pH 7.1.

e. Beef extract - 0.3 per cent beef-extract broth.

f. Brain-heart-gelatin - Pharmagel A, 2 grams, Na_2HPO_4, 0.16 gram brain-heart infusion, 37 grams, distilled water q.s. to 1000 ml.

exponential die-away of organisms in the presence of toxic agents and is determined by the following expression:

$$k = \frac{\log n_0 - \log n_1}{t} \qquad (5)$$

TABLE XLVIII

MEASUREMENT OF VISCOSITY OF VARIOUS IMPINGER MEDIA†

Water	Gelatin	Brain-Heart Gelatin	Gelatin, DOW AF, Sterilized	Gelatin, DOW AF, Sterilized, Air Bubbled Through	Brain-Heart Gelatin, DOW AF, Sterilized	Brain-Heart Gelatin, DOW AF, Sterilized Air Bubbled Through
9.0	10.1	11.1	9.3	9.2	10.6	10.4

† Measurements made with Oswald-Fenske pipette, 6-ml volume. Average of 10 determination, 5 with pipette No. 148 and 5 with pipette No. 149. All measurements made at room temperature, 25° C.

where n_0 = the original number of organisms, and

n_1 = the final number of organisms after lapse of time t (t expressed in minutes).

This expression for k differs from the K used to describe physical phenomena which obey an exponential die-away pattern

$$n_1 = n_0 e^{-Kt}. \tag{6}$$

Rearranging,

$$K = \frac{(\log n_0 - \log n_1)(2.3)}{t} \tag{7}$$

where the terms are as described above.

The relation between k and K is merely the conversion value between $\log_e$ or $\log_{10}$, or

$$K = (k)(2.3). \tag{8}$$

The term K is also related to the dimensions of an enclosed space and the particles within that space

$$K = \frac{v}{h} \tag{9}$$

where v = rate of fall of the particle in cm/min, and

h = height of the enclosed space in centimeters.

The loss of viable air-borne organisms can be expressed in numerically similar terms by converting mechanical rates of loss to expressions of k; these values can then be used with the biological values. The term k or k_t is used in

this report to denote the sum total of all such effects

$$k_t = k_d + k_f \tag{10}$$

where k_d = rate of loss due to death, and

k_f = rate of loss due to fall-out.

For any enclosed space:

$$k_f = \frac{v}{(h)(2.3)} \tag{11}$$

and for the 4-foot chamber

$$k_f = (v)(0.00357). \tag{12}$$

Velocity of fall and diameter of particles have been handled with a simplification of Stokes' equation:

$$v = 0.18\ d^2$$

and

$$d = \sqrt{5.55\ (v)} \tag{13}$$

where d = diameter of the particle in microns.

The aerial concentration of air-borne organisms and the number settling on plates during dynamic runs have been related by converting the numbers falling on a single Petri dish by the factor 15.2, thus converting to numbers falling per minute per 1,000 cm^2. The rate of fall is thus:

$$v = \frac{\text{numbers per 1000 cm}^2/\text{min}}{\text{aerial concentration/l.}} \quad . \tag{14}$$

The calculation of death rates from data obtained from dynamic runs is

$$k = \frac{\log \text{in} - \log \text{out}}{t} \tag{15}$$

where in = inlet concentration,

out = outlet concentration,

t = 3.5 minutes for single aerosol cylinder, or

t = 7.0 minutes for the double aerosol cylinder, or

t = 5.0 minutes for the main aerosol chamber.

In the main aerosol chamber, where natural settling can occur, the calculated value of k_f is subtracted from the value of k_t to obtain the net k due to death.

The data from static runs were plotted on semi-log paper, the best line was located visually, and k_t was determined for a 10-minute period.

Because k is an expression of the rate of die-away, it is readily converted into specific death values. Thus, the following expressions for the time required to kill various percentages of initial numbers of organisms can be obtained from k values (or conversely k can be obtained from them).

$$\text{Time required to kill 50 per cent} = t_{50} = \frac{1.301}{k}$$

$$\text{Time required to kill 90 per cent} = t_{90} = \frac{1}{k}$$

$$\text{Time required to kill 99 per cent} = t_{99} = \frac{2}{k}$$

$$\text{Time required to kill 99.9 per cent} = t_{99.9} = \frac{3}{k}$$

No intrinsic significance is attached to the expression k. It is actually the negative slope of the regression line obtained by plotting the logarithms of numbers surviving against time. As such it is simply a rate of death. The various expressions given above for time required to effect either 50 or 99.9 per cent kill are probably more meaningful; the former to the physical sciences, the latter to bacteriologists. However, mathematically the term k is extremely easy to use and serves many valuable functions which can not be served by these expressions.

D. Miscellaneous

1. A Note on the Possible Relation between Aerial Disinfectants and Insect Repellents

It was recently noted that the compound which had been selected as a model for the study of nonhygroscopic compounds as aerial disinfectants, 2-ethylhexanediol (1,3) is widely known as an insect repellent. It is referred to as "Rutgers 612" or simply "612" having replaced dimethyl phthalate as the insect repellent of choice. The phthalate esters possess no in vitro toxicity towards bacteria and exhibit no power as aerial disinfectants However, 2-hydroxypropyl cyclohexanol is used as an insect repellent, and it was reported by Grün† to have aerial disinfectant properties equal to triethylene glycol. Recourse to the literature revealed that the ideal insect repellent has many characteristics in common with the ideal aerial disinfectant. Both should have low, but definite vapor pressures under conditions of use; be nontoxic to humans; have no objectionable

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† Grün, Zbl. Bakt. I. Orig. 162, No. 3, 213 (1955).

odor; and be as low in cost as possible. The only possible difference in characteristics between the two is that certain classes of insect repellents are listed as water insoluble. The exact truth of this is unknown because the extremely limited water solubilities of many of the most effective bactericidal agents indicate that the term "insoluble" is a relative one.

It was found that the Bureau of Entomology and Plant Quarantine, Agricultural Research Administration of the U. S. Department of Agriculture, has been supporting a continuing program of screening candidate compounds as insect repellents. In various publications resulting from this program, the classes of compounds of greatest interest have been listed. These are esters, diols, N. N-dialkylamides, substituted mandelic acids, and esters of 1-hydroxy cyclohexane†.

In future studies of aerial disinfectants, the close similarity between the desired characteristics for insect repellents and aerial disinfectants should be considered. Using the approach set forth in this report of relating *in vitro* toxicity to aerial killing power, evaluation of potential insect repellents as aerial disinfectants could be carried out with a minimum of effort.

2. Test of Small Ultraviolet Ozone Lamp

Currently, small mercury vapor lamps are being sold for the purpose of "eliminating kitchen odors." These lamps have a low pressure and lowwattage, and, when operating, they give off a perceptible odor of ozone. One of these units being available, a few tests were made to determine the possible aerial disinfectant powers of the combined effects of ozone and ultraviolet irradiation. The unit tested was rated at 20 watts' power consumption and consists of a transformer

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† *J. Organ. Chem.* 19, 485 (1954).

and a small, mercury-vapor bulb. The bulb is labeled "Westinghouse Odorout-794H."

A test was made with the above unit suspended in the center of the main aerosol chamber (68°F, 60 per cent relative humidity). Comparison of the inlet and outlet concentration of air-borne viables during dynamic runs with the unit operating and then with the unit not operating showed than the unit effected a net death rate equivalent to a k of 0.12.

In order to distinguish the difference between the possible effect of the ozone produced and the ultraviolet irradiation, the unit was positioned in the inlet to the aerosol cylinders in such a manner that all the diluting air going through the cylinder passed over the unit, prior to the admixture of this diluting air with the bacterial aerosol. In this manner, it was assumed that any ozone produced by the lamp would reach the bacterial aerosol, but that the bacteria would be shielded from the radiation. Under these conditions, no net kill was found due to the lamp unit.

Therefore, it was concluded that the "Odorout" lamp does possess some effectiveness against air-borne bacteria, and that this is entirely due to the ultraviolet radiation and not to the ozone produced by the lamp.

3. Test for a Possible Two-Stage Die-Away

The work of various investigators has indicated that, under certain conditions and with certain organisms, air-borne bacteria may exhibit a two-stage die-away. That is, an early very rapid death rate is shown, followed by a more moderate rate of death. This situation could probably not exist under the conditions under which the present studies were made, namely, the aging of the bacterial aerosol in the prechamber prior to use in experimental studies.

However, in order to verify or to deny this, a few tests were made in the double aerosol cylinders (two cylinders in series). The tests were carried out on the assumption, if S. marcescens (E-R/O) did exhibit a two-stage die-away, that the death rate in the first cylinder should be perceptibly greater than that exhibited in the second cylinder.

Tests made under conditions of room temperature indicated that there was no difference between the k values obtained from data taken from the two cylinders. However, the k values were so small that a small difference could have escaped detection. Therefore, two tests were made at elevated temperature with the double cylinders located in an insulated box. Under these conditions, the critical-orifice, liquid-impinger samplers were outside the insulated box and were held at 70° F. The results of these tests showed no difference between the k values obtained from the data taken from either cylinder. The values were taken from air at 100° to 104° F and 13 per cent relative humidity; the first k was 0.133, and the second 0.150. At 20 per cent relative humidity, the corresponding values were 0.10 and 0.11.

4. Notes on the Efficiency of Air Samplers

a. Absolute Efficiency. It has been impossible to determine the absolute efficiency of the impinger samplers under the conditions of operation. The magnitude of the total numbers of air-borne particles produced by the atomizer and the losses experienced in the prechamber combine in such a fashion that a small error in the estimation of the losses in the prechamber can result in a large error in the estimation of numbers leaving the prechamber.

The large body of data available from the screening runs were assembled, and it was estimated that the recovered maximum concentration of viable air-borne

organisms issuing from the prechamber was 370 x 10^4 per liter of air. (See Chapter V-C on the response to a sudden change in relative humidity.) This figure is obtained by calculating the maximum numbers per liter recovered after dilution with room air, and estimating the original concentration of bacterial aerosol issuing from the prechamber, using the factors involved in diluting 6.4 liters of prechamber air with 21.9 liters of room air to yield a total of 28.3 liters (1 ft^3).

The average consumption of fluid culture by the atomizer during the screening tests was 7.37 ml/hr or 0.123 ml/min. The average concentration of viable organisms per milliliter of culture fluid was 80 x 10^7/ml. Thus, 9.84 x 10^7 of bacteria was suspended in the prechamber during each minute of operation, and corrected to a basis of an original culture count of 100 x 10^7/ml (as with the other data handled), the number is 12.3 x 10^7.

The actual concentration of the aerosol issuing from the prechamber was thus 19.3 per cent of the maximum possible number if no losses occurred in the prechamber. The prechamber is a 36.8-cm cube, having a volume of 50 liters. The theoretical retention time of this cube at a flow of 6.4 liters per minute is 35 minutes. The $\underline{k}_t$ values taken for such a chamber under conditions of operation would be equal to the difference between the $\log_{10}$ of the number entering and the $\log_{10}$ of the numbers leaving divided by 35. In this instance, $\underline{k}_t$ would be 0.02, as calculated from the data for the estimated numbers produced by the atomizer and the observed numbers leaving the prechamber.

The value for $\underline{k}_t$ calculated above must be the sum of the $\underline{k}$ values for losses due to fall-out and to death. In the prechamber, if the majority of the particles are 3.0 microns in diameter, the value for $\underline{k}_f$ would be 0.019. Under the

conditions of operation, the k due to death alone is certainly not greater than 0.008. Therefore, the sum of these two values is equivalent to a calculated k_t of 0.027. This is greater than the observed k_t of 0.020 determined above. In other words, the observed recoveries were of the order of 19 per cent, and the calculated recoveries would be of the order of 11 per cent. It should be noted here that if plain-gelatin fluid had been used in the samplers instead of enriched fluid (at a comparative effectiveness of 1:2), the comparison would have been more favorable; a recovery of 9.7 per cent as compared to a calculated output of 11.4 per cent of the maximum. This very fact indicates the difficulty of obtaining an exact evaluation of the absolute efficiency of air samplers under the conditions of these experiments. The original numbers are so great that a small error in estimating the losses in the prechamber results in a very large error in the calculation of the output of the prechamber. Thus, if the prechamber is not operating as a diffusion chamber and the actual retention time were 25 minutes instead of 35, the observed k_t for the prechamber would be 0.028, and all values would compare with each other in a favorable manner.

b. Comparison of Various Samplers. A limited study was made of the effectiveness of various samplers for air-borne bacterial particles. The work reported in this section was done using plain-gelatin fluid in the samplers and with an undifferentiated culture of S. marcescens. However, on a purely comparative basis, observations were made on the efficiency of the following samplers: sieve samplers, singly and in series (at 1 cfm); Greenberg-Smith samplers (100-ml gelatin fluid, at 1.5 cfm); midget impingers (5-ml gelatin fluid at 0.3 cfm); and nozzles, 2.3-mm diameter (same as Greenberg-Smith) operated either in 1-qt. milk bottles or in Greenberg-Smith tubes (200-ml gelatin fluid) at 1.5 cfm. The

last sampling arrangement was made in order to determine whether the close location of the plate in the Greenberg-Smith sampler was the cause of losses. Such an arrangement would represent scrubbing action only.

In order to use the sieve samplers outside the chamber, a funnel was attached, as shown in Figure 19. The other samplers used are shown in Figure 20.

Sampling was accomplished by withdrawing aerosol samples from the main aerosol chamber during dynamic runs. In all cases, the result obtained from the 1-liter-per-minute, critical-orifice, liquid impingers was taken as 100 per cent. All runs were made at 68° to 70°F and at 60 per cent relative humidity. The data obtained are shown in Table XLIX.

TABLE XLIX

COMPARISON OF VARIOUS SAMPLERS WITH CRITICAL-ORIFICE, LIQUID IMPINGERS AT 68° F AND 60 PER CENT RELATIVE HUMIDITY, PLAIN-GELATIN FLUID IN ALL LIQUID SAMPLERS

Per Cent Recovery of Viable Organisms From Aerosol Chamber						
	Sieve Samplers					
1.0 l./min Critical-Orifice, Liquid Impinger	Single	Sum of 5 in Series	Greenburg-Smith	Midget Impinger	1.5 cfm Nozzle in G-S Tube	1.5 cfm Nozzle in Milk Bottle
100	38	52	50	43	35	75

The only sampler that showed any promise as the result of the studies detailed in Table XLIX was the 1.5-cfm nozzle operated in the milk bottle. However, further tests at low relative humidities (45 and 36 per cent) showed a reduction in efficiency to about 50 per cent of the 1-liter-per-minute, critical-orifice, liquid impingers. Presumably the reduction in the size of the airborne particles effected by lowering the relative humidity was responsible for this reduction in efficiency.

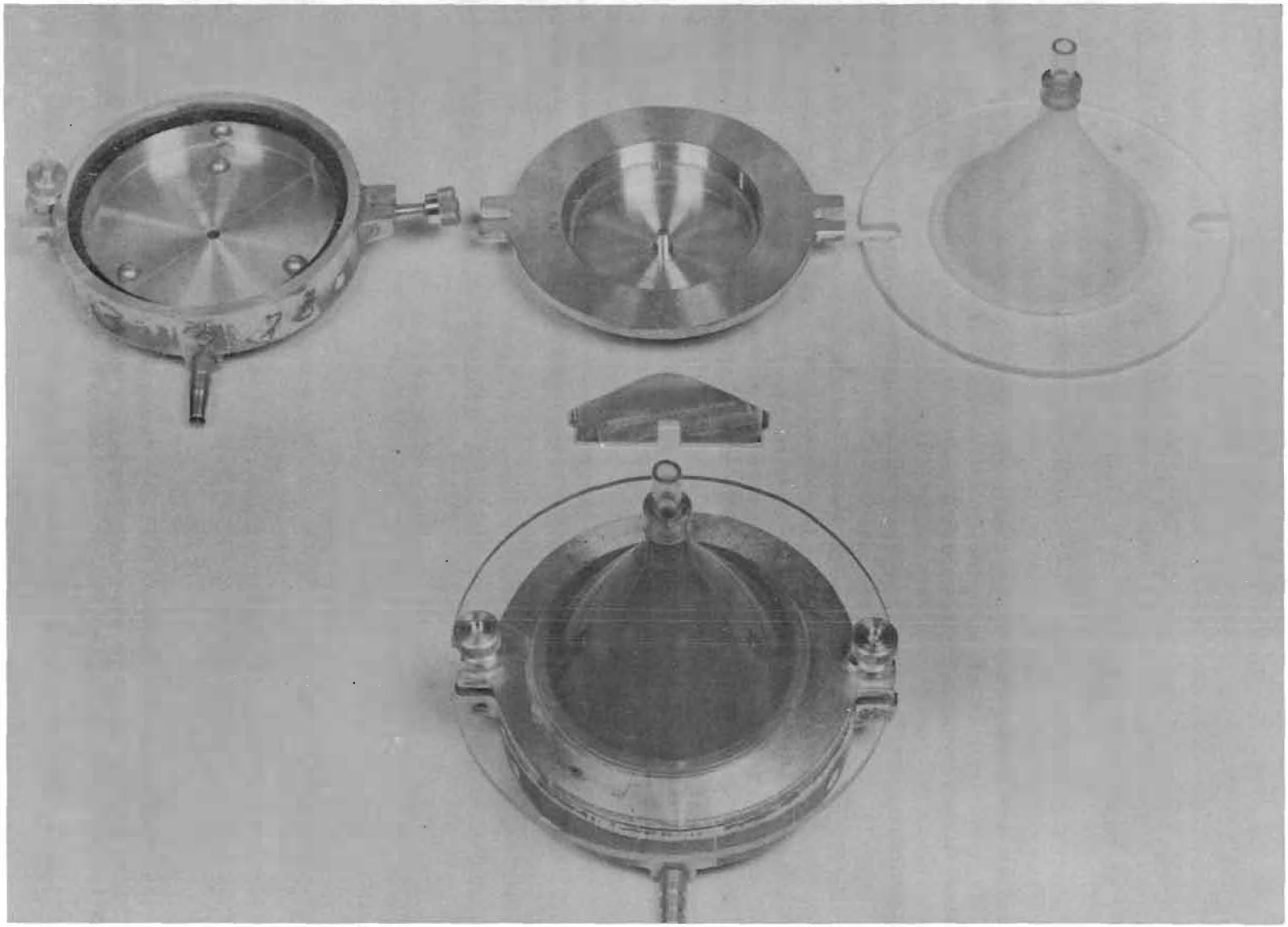

Figure 19. Sieve Sampler and Funnel Attachment.

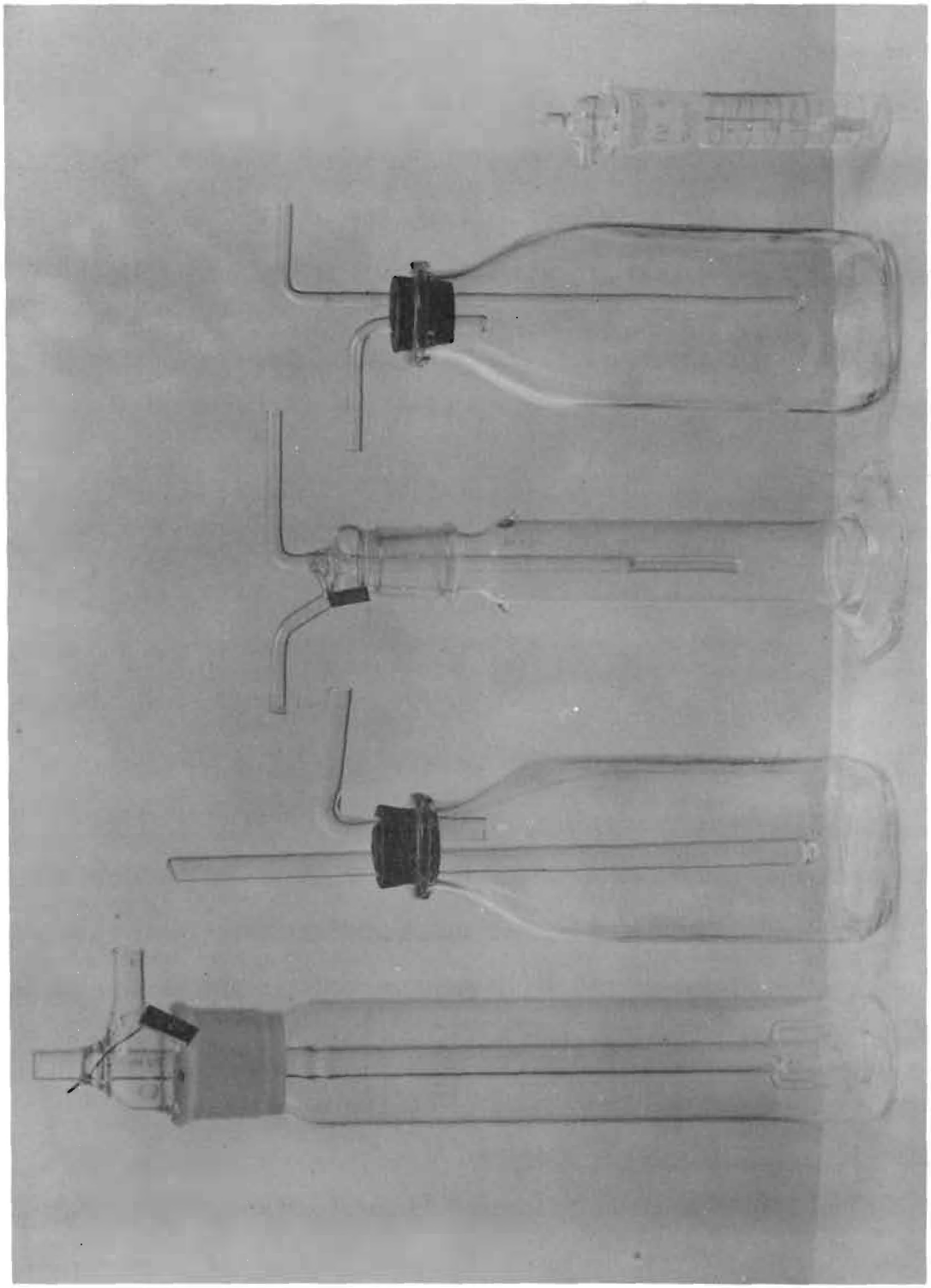

Figure 20. Various Air Samplers.

The results of these studies were so unfavorable that no further attempt was made to use any sampler other than the critical-orifice, liquid impingers for routine work.

5. The In Vitro Toxicity of Benzoic Acid

It was planned to employ benzoic acid in the screening tests, but, time did not permit this evaluation. However, the *in vitro* toxicity of benzoic acid against *S. marcescens* (E-R/O) was determined, and the data are shown in Table L.

TABLE L

IN VITRO TOXICITY OF BENZOIC ACID AGAINST *S. marcescens* (E-R/O) AT 20° C

Per Cent of Aqueous Saturation of Compound	Net k Due to Death
50	0.062
55	0.13
70	0.19
80	0.30
100	0.59

It is predicted on the basis of the *in vitro* toxicity of benzoic acid that, at relative humidities above 60 per cent and fractional aerial saturation of 70 per cent or greater, this compound would exhibit definite activity as an aerial disinfectant. Aerial saturation at 20° C is estimated to be 0.3 mg/ft^3 (vapor pressure at 20° C of 0.0016 mm Hg).

6. Counters for Air-borne Particles.

A considerable amount of time and effort was expended on attempts to estimate the concentration of air-borne particles by means of light-scattering

devices. A variety of optical systems were used. However, none of the attempts were sufficiently successful to warrant use. During the course of these studies, a few tests, the results of which showed some promise, were made using light in the near ultraviolet region. These studies were made on the assumption that better resolution could be obtained with light of shorter wavelength than the visible. The optical system and light source of a Coleman photofluorometer were arranged so that the photoelectric tube of the photoflurometer picked up the right angle scattering from the bacterial aerosol which was passed through the ultraviolet beam. Under these conditions, the output of the phototube showed a quantitative response to varying concentrations of aerosol. However, there was no discriminator circuit, and there was no way of telling whether the system was equally affected by particles of various sizes. It was thought that a study using light of shorter wavelengths in a particle counter might prove fruitful, but the study was not pursued further than this conjecture.

7. Development of a Thermal-Precipitator Sampler

In the early stages of the work herein reported, various efforts were made to develop a more efficient and useful sampler for use in aerobiological studies. Because the development of samplers was not a primary objective of the project, such studies were limited in nature. However, as a result of these efforts a new type of thermal-precipitator sampler was developed, which successfully operates at flow rates as high as 400 cc/min as compared to 5 cc/min for previous thermal precipitators. This information was published in a short note†.

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† Science 116, 368 (1952).

The full possibilities of this sampler were not exploited in connection with the present project because the major effort should be directed toward the more primary objectives of the project concerning the behavior of bacterial aerosols. However, in the work carried out by other groups at Georgia Tech, further work has been done with this thermal precipitator, which shows that this sampler is a valuable instrument for the study of air-borne particles of all kinds. A commercial instrument based on the principles developed in the course of this project is currently being marketed by a local company (Roy S. Martin Company) and is shown in Figure 21.

Figure 21. Commercial Model Thermal Precipitator.